U.S. Fish and Wildlife Service
Columbia River Fisheries Program Office

Eagle Creek Hatchery-Wild Steelhead Ecological Interactions:
Comparative abundance, growth, migration behavior and survival of winter steelhead in upper Eagle and North Fork Eagle Creeks

2010-2015 Final Report

Maureen Kavanagh, Doug Olson, Brian Davis, Jen Poirier, and Steve Haeseker

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Columbia River Fisheries Program Office
Vancouver, WA 98683

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On the cover:

Juvenile winter steelhead in Eagle Creek, in the Clackamas River drainage of Oregon. Photograph by Maureen Kavanagh.

Disclaimers:

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Eagle Creek Hatchery-Wild Steelhead Ecological Interactions:
Comparative abundance, growth, migration behavior, and survival of winter steelhead in upper Eagle and North Fork Eagle Creeks, 2010-2015 Final Report

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Abstract. Eagle Creek National Fish Hatchery spawns and rears juvenile coho salmon (*Oncorhynchus kisutch*) and winter steelhead trout (*O. mykiss*) that are released into Eagle Creek within the Clackamas River basin, Oregon. Previous investigations on the ecological and genetic impacts of hatchery winter steelhead in Eagle Creek during 2005-09 indicated that in some years natural production was influenced by naturally spawning hatchery fish. Using Passive Integrated Transponder (PIT) tag technology, we initiated a follow-up study to compare the abundance, growth, survival, and migration behavior of hatchery and wild winter steelhead in Eagle Creek, 2010-15. We compared an area of natural production most influenced by hatchery fish (upper Eagle Creek) to an area with less hatchery influence (North Fork Eagle Creek). Our hypotheses were that abundance, growth, and survival of naturally produced winter steelhead in upper Eagle Creek would be negatively impacted by naturally spawning hatchery fish. We were also interested in whether the migratory behavior of naturally produced fish in the two streams were similar to each other or exhibited patterns similar to hatchery fish. Problems were encountered with some of our sampling techniques and sample sizes were often low; nevertheless, we could not conclude that freshwater abundance, growth, survival, and migration behavior of naturally produced winter steelhead in upper Eagle Creek were negatively impacted by naturally spawning hatchery winter steelhead when compared to North Fork Eagle Creek. Juvenile and adult winter steelhead from upper Eagle Creek performed as well as, or better than, fish from North Fork Eagle Creek. Even though juvenile hatchery winter steelhead were found residualizing in upper Eagle Creek but not in North Fork Eagle Creek, relative growth and abundance of juvenile winter steelhead were higher in upper Eagle Creek. No significant differences were found in over-summer survival of juveniles or in survival to adult. A greater proportion of migratory juveniles from upper Eagle Creek were detected in the lower Columbia River compared to North Fork Eagle Creek. The migration behavior for naturally produced winter steelhead from upper Eagle Creek and North Fork Eagle Creek were more similar to each other than to hatchery fish. Genetic samples from 2005, 2006, and 2007 were examined by Abernathy Fish Technology Center along with samples collected in 2010 and 2011 in order to estimate the contribution of hatchery and wild steelhead to natural production in each of the study sites. While both streams had evidence of hatchery-wild admixture, in three out of five years studied, the North Fork Eagle Creek had less hatchery influence than upper Eagle Creek. When making conclusions about genetic data, it was important to interpret the genetic impact of the hatchery in the context of the timeframe it was sampled. The hatchery influence from genetic samples collected in 2007, 2010 and 2011 was significantly higher in upper Eagle Creek than in North Fork Eagle Creek; however, in 2005 the hatchery influence was higher in North Fork Eagle Creek (no difference in 2006). Our findings support the need for periodic evaluation of the hatchery program in Eagle Creek to ensure parameters in the Hatchery and Genetic Management Plans and Section 7 Endangered Species Act Biological Opinion are met.

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Introduction

The U.S. Fish and Wildlife Service operate Eagle Creek National Fish Hatchery, with reimbursable funding provided by NOAA Fisheries through the Mitchell Act. The hatchery began operations in 1956 and production has changed over time depending on available funding, evaluation results, and co-manager agreements (USFWS 2007). The hatchery has a history of producing fall and spring Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), and steelhead trout (O. *mykiss*). During the period 1990-2007, the number of juvenile hatchery fish annually released into Eagle Creek averaged 836,325 coho salmon and 176,000 winter steelhead. For brood years 2009-13 the planned juvenile hatchery production was 350,000 coho salmon and 100,000 winter steelhead for release into Eagle Creek. Coho salmon are also produced at the hatchery for transfer to Tribal fish restoration programs upstream of Bonneville Dam, but within Eagle Creek the hatchery has been classified as a segregated brood stock for harvest (USFWS 2007). Naturally produced juvenile coho salmon and winter steelhead are also found in Eagle and North Fork Eagle Creeks (Kavanagh et al. 2009).

Naturally produced “wild” winter steelhead in Eagle Creek of the Clackamas River watershed in northwestern Oregon, are a population component of a Distinct Population Segment having *Threatened* status under the Endangered Species Act (ESA; 63 FR 13347, March 1998). To partially address ecological and genetic interactions between hatchery and wild winter steelhead in Eagle Creek, a five-year evaluation was completed by the U.S. Fish and Wildlife Service in July, 2009 (Kavanagh et al. 2009). The results from that study identified the North Fork Eagle Creek as having the least amount of hatchery influence (based on radio telemetry, genetic analyses, snorkel/electrofishing surveys, and spawning ground surveys), whereas upper Eagle Creek (Figure 1), in particular, the section of stream between the middle ladder and hatchery (rkm 14-21), was identified as the area having the greatest hatchery influence. Other interesting findings from Kavanagh et al. (2009) and Brignon et al. (2012) pertain to juvenile rearing densities (fish/m^2). The authors found that age-1 juvenile winter steelhead were distributed throughout Eagle Creek and North Fork Eagle Creek but at lower densities than age-0 fish. For age-0 juvenile winter steelhead, upper Eagle Creek had the highest density and abundance compared to other areas in Eagle Creek and North Fork Eagle Creek. The authors also found the highest density of residual hatchery juvenile steelhead in upper Eagle Creek and none were found in North Fork Eagle Creek.

One of the recommendations in Kavanagh et al. (2009) was to compare the productivity in North Fork Eagle Creek to that in upper Eagle Creek using spawner-recruitment and smolt-to-adult recovery rates to assess the impact of naturally spawning hatchery fish on the fitness and productivity of the natural population. A difference between wild spawner-recruitment rates in upper Eagle Creek and North Fork Eagle Creek could indicate that there are negative effects of naturally spawning hatchery fish on the wild population in upper Eagle Creek. Chilcote (2003) found that naturally spawning hatchery fish negatively impacted population productivity, overall fitness of wild fish, and reduced the number of recruits by one-third when hatchery fish comprised 30% or greater of the spawning population. Other studies (Araki et al. 2007; Lynch and O’Hely 2001) have shown that the progeny of naturally spawning hatchery fish are less fit and have lower adult survival than wild fish. Based on these findings, we hypothesize that freshwater productivity, survival, and spawner-recruitment rates in upper Eagle Creek are negatively impacted by naturally spawning hatchery fish.

To investigate our hypothesis, we compared several indices of productivity, including growth, relative abundance, young-of-year to yearling smolt survival, and smolt-to-adult survival in North Fork Eagle Creek and upper Eagle Creek. Unfortunately, the number of wild steelhead smolts could not be estimated because of problems with our lower Eagle Creek detection site (see Methods). We therefore modified our sampling plan to address the following objectives: 1) Estimate the relative abundance, growth, and survival of juvenile winter steelhead in upper Eagle Creek and North Fork Eagle Creek, 2) Estimate juvenile-to-adult survival of hatchery and wild winter steelhead in upper Eagle Creek and North Fork Eagle Creek, 3) Determine juvenile downstream and adult upstream migration behavior of hatchery and wild winter steelhead in the Eagle Creek basin, and 4) Determine the genetic contribution to natural production of hatchery and wild winter steelhead in Upper Eagle Creek and North Fork Eagle Creek. This final report summarizes our 2010-2015 field investigation of assessing the ecological and genetic impacts of a segregated hatchery program on the productivity, behavior, and survival of naturally produced winter steelhead in upper Eagle and North Fork Eagle Creeks.

Study Area

Study Site- Eagle Creek, one of the major tributaries to the lower Clackamas River, originates in the Mount Hood National Forest and flows northwest to its confluence with the Clackamas River at river kilometer 26 (Figure 1). The main-stem of Eagle Creek is managed for both natural and hatchery production of salmon and winter steelhead. Eagle Creek National Fish Hatchery (NFH) is located at river kilometer 20 of Eagle Creek, approximately 1 kilometer below the upper falls which are an impassable fish barrier. The hatchery operates three fish ladders to facilitate upstream adult fish passage. One ladder is located at the lower falls (rkm9) below the confluence of Eagle Creek and North Fork Eagle Creek, and one is located at the middle falls (rkm 14). A third ladder is located at the entrance to the hatchery (rkm 20) and is used to collect brood stock and surplus hatchery fish. In addition to the planned hatchery production and release of approximately 100,000 juvenile winter steelhead and 350,000 juvenile coho salmon into Eagle Creek, a spring Chinook salmon program of 240,000 fish was initiated with Oregon Department of Fish and Wildlife in 2013 for release in 2015. Kavanagh et al. (2009) and USFWS (2007) provide additional information on the hatchery program.

North Fork Eagle Creek flows into Eagle Creek at river kilometer 11, just upstream of the lower fish ladder (Figure 1). No juvenile hatchery fish are released into the North Fork Eagle Creek. North Fork Eagle Creek is managed as a natural production area for salmon and winter steelhead in the Eagle Creek basin.

Our study objectives were to compare the growth, behavior, and survival of winter steelhead in two study sites, upper Eagle Creek and North Fork Eagle Creek (Figure 1). The lower boundary of the upper Eagle Creek study site was approximately 100 meters upstream of the middle falls fish ladder located at rkm 14. The upper boundary of the study site was at the upper falls (rkm 21). The total distance between the lower and upper boundaries was approximately 6 kilometers.

The upper Eagle Creek study site was sub-divided into 30, 200-m reaches for sampling. The lower boundary of the North Fork Eagle Creek study site was at the confluence with Eagle Creek, and the upper boundary was 12 rkm upstream, approximately 3 rkm downstream from the headwaters of the North Fork. The North Fork Eagle Creek study site was sub-divided into 58, 200-m reaches for sampling.

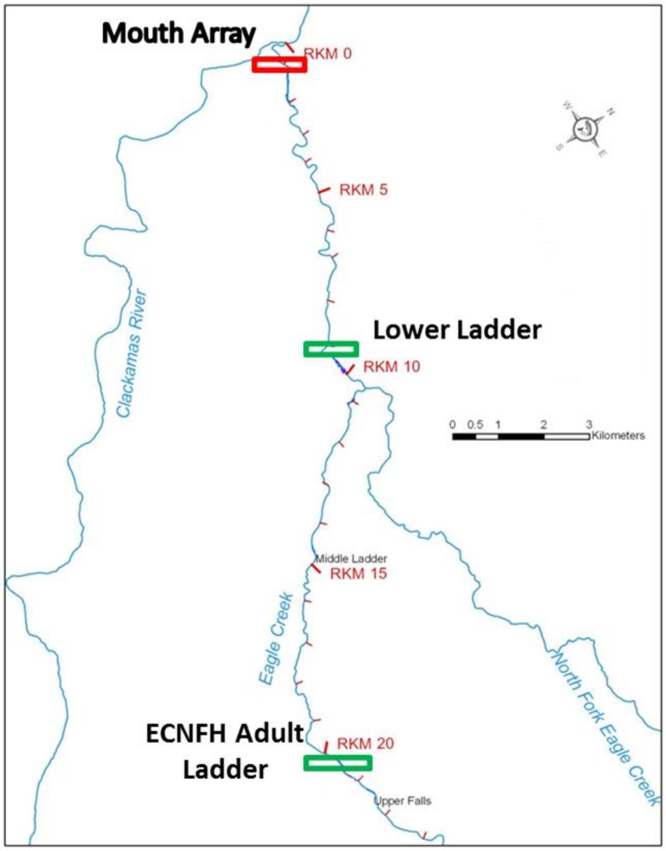

Figure 1. Map of Eagle and North Fork Eagle Creek study area. The lower and upper boundaries of the Eagle Creek study area extended from the middle ladder (rkm 14) to the upper falls above Eagle Creek National Fish Hatchery (rkm 20). The lower and upper boundaries of the North Fork study area extend from the confluence of North Fork Eagle Creek and Eagle Creek, just above the lower fish ladder on Eagle Creek, to just below the headwaters of North Fork Eagle Creek (rkm 12).

Methods

Fish Sampling and PIT Tagging

Marking- To achieve our objectives, juvenile fish from each of the study sites were marked using Passive Integrated Transponder (PIT) tags. Our goal was to PIT tag 1,500 juvenile steelhead in upper Eagle Creek and 1,500 in North Fork Eagle Creek each year for three years. This tagging level was chosen as a balance between maximizing the number of fish to tag each year in order to achieve our objectives, being logistically feasible, and minimizing impact to wild fish. To reach the tagging goal, upper Eagle Creek (30 reaches) and North Fork Eagle Creek (58 reaches) study areas were sampled throughout July to early August 2010-12. Sampling began each year in the North Fork reaches, and then sampling efforts were alternated between the two study areas every one to two weeks. For each 200-m reach, a single upstream electrofishing pass was made without blocknets using a Smith-Root backpack electrofisher (Figure 2). During electrofishing, we targeted steelhead for capture that visually appeared >74 mm in length. At the end of a reach, captured steelhead were sedated with tricaine methanesulfonate (MS-222), measured (fork length, mm) and weighed (g). Scales were also collected from a subset of the sampled population for age determination. Steelhead >74 mm were tagged with unique Passive Integrated Transponder (PIT) tags (Digital Angel: TX1411SST, 12.5 mm x 2.1 mm, 0 .201 g). PIT tags were inserted into the ventral body cavity of the fish using a hypodermic needle following methods described in the PIT Tag Marking Procedures Manual (CBFWA 1999). Tagged fish were allowed to recover in an aerated five gallon bucket of stream water before being returned to the reach where captured. Other non-target fish species incidentally captured in a reach were enumerated and released.

Additionally, 1500 age-1 hatchery winter steelhead at Eagle Creek National Fish Hatchery were PIT tagged and released in April 2011 and 2012 to estimate and compare survival and migration behavior to wild fish. Hatchery juvenile steelhead that did not leave Eagle Creek and residualized during the summer were also captured while electrofishing and were PIT tagged.

Figure 2. Electrofishing a sample reach in Eagle Creek.

Recapture- Recaptures of tagged fish were used to estimate relative abundance, growth, and summer survival of juvenile steelhead. For sample years 2010-12, we began recapturing fish in upper Eagle and North Fork Eagle Creeks in mid-August to early September, approximately one month after initial tagging. For all years, 15 reaches in upper Eagle Creek and 13 reaches in North Fork Eagle Creek were randomly selected for recapture. A single upstream electrofishing pass, without blocknets, was made in the selected 200-m units to capture fish. Captured steelhead were anaesthetized, physically examined for the presence of a mark or tagging scar, and scanned for a PIT tag using a portable PIT tag detector (i.e. FS 2001-ISO, or Oregon RFID GES3S). If a PIT tag was present, the tag information was stored electronically in a data file on the PIT tag reader and/or manually recorded on a data sheet. Recaptured fish were measured and weighed to collect information on growth rate between sampling periods.

In years where we were unable to meet our goal of PIT tagging 1500 steelhead in both upper Eagle and North Fork Eagle Creeks (2011 & 2012), untagged fish captured during the recapture period were measured, weighed and implanted with PIT tags.

Stationary PIT Tag Antennas in Eagle Creek and Lower Columbia River Detection Sites

Eagle Creek Mouth Detection Array– A PIT tag antenna array was installed at the mouth of Eagle Creek to detect out-migrating tagged juvenile winter steelhead (Figure 1). Detections were used in juvenile survival analyses as well as to monitor the migration behavior of juvenile fish. The Eagle Creek Mouth (ECM) detection array was installed in July 2010 and was located in Eagle Creek on private property approximately one kilometer upstream from the confluence of Eagle Creek and the Clackamas River. The site consisted of four antennas operated with a single FS1001M multiplexing transceiver (MUX). The four antennas were positioned in line spanning perpendicular to the stream channel. The ECM detection array spanned approximately 95% of the stream width profile during base flow. PIT antennas were constructed out of 10.2 cm (4") schedule 80 PVC with 1.9 cm (¾") schedule 40 PVC and insulation foam internally supporting 14-gauge, 8-strand ribbon cable. The final inductance values of the antennas ranged from 224 to 260 µH, and the final capacitance values ranged from 5.56 to 6.25 nF depending on antenna type and location. The Eagle Creek MUX, stored in an aluminum lockbox, was powered off grid using an AC to DC power adapter and was configured to upload daily interrogation files to a laptop computer using PTAGIS Minimon software. Site checkups including debris removal, manual tuning, and data downloads were conducted year round on a weekly basis while the site was operational.

The original ECM antenna design included four antennas, each of them 6.1 meters long and ranged from 0.5 to 1 meter in width. Antennas were anchored to the stream bottom using platypus earth-anchors with 5 mm stainless steel cable, and polypropylene straps rated to 680kg (Figure 3). Using a drive rod and sledge hammer, earth-anchors were driven 0.5 meters into the stream substrate leaving a loop exposed at the surface for attachment. Anchors were secured with a manual high-lift jack to rotate and lock the anchor head in place. Each antenna was mounted to the stream bottom with a minimum of six anchoring points. Only the upstream portion of the antenna was fixed to the stream bottom with the anchoring system working as a hinge so the downstream end remained buoyant and free to fluctuate with stream flow.

Figure 3. Eagle Creek Mouth (ECM) detection array and stream bottom anchoring system during base flows.

After a flood event in late January 2011, the original ECM antennas were destroyed. Four new antennas were built and installed in early March 2011 at the same location. The new antennas were shorter in length (3.1 meters) and width (0.6 meters) and were securely anchored to the stream bottom on both the upstream and downstream ends (i.e. pass over configuration). A flood and debris flow in November 2011 destroyed these new antennas (Figure 4). The ECM antennas were not rebuilt and the site was decommissioned.

***Lower Ladder Antenna*–** A PIT tag antenna array was installed in the lower fish ladder at rkm 9 on Eagle Creek. This antenna array was used to monitor juvenile migration behavior as well as detect returning tagged adults. The antenna was installed in December 2011 and was operated through April 2015. The site consisted of a rectangular, pass through antenna built to encompass the ladder opening (0.4 x 1.3 m) [Figure 5]. The PIT antenna was constructed using 5.1 cm (2”) schedule 80 PVC with internal schedule 40 PVC and insulation foam to support the 8 strand ribbon cable. Antenna configuration and tuning was accomplished using a FS2001-ISO portable reader (i.e., cheese block) and external tuning box. Final antenna inductance was 303 µH with a capacitance of 4.03 nF. The FS2001 reader was housed in an aluminum lockbox secured inside the ladder and was powered by two 12V DC batteries connected in parallel. Batteries were exchanged once per week to maintain a continuous power supply. Data files with individual PIT codes and corresponding date/time stamps were downloaded weekly from the reader using a file transfer application (i.e. HyperTerminal or PuTTY).

Figure 4. Eagle Creek Mouth (ECM) site during fall 2011flood.

Figure 5. PIT antenna inside the lower fish ladder on Eagle Creek.

***Hatchery Ladder Antenna*–** A PIT tag antenna array was also installed in the hatchery ladder to monitor juvenile migration behavior as well as detect returning tagged adults. The Eagle Creek hatchery detection antenna was installed in January 2012 and operated through March 2015 and was located approximately 3 meters upstream from the fish ladder entrance at Eagle Creek National Fish Hatchery (rkm 20) [Figure 6]. The site consisted of a single, rectangular pass through antenna (0.7 x 2.8 m), with a FS1001M multiplexing transceiver. The antenna was constructed using 5.1 cm schedule 80 PVC with schedule 40 PVC and insulation foam internally supporting two strands (i.e. 6 loops) of CAT 6 ethernet cable. Final antenna inductance was 223 µH, with a capacitance value of 16.24 nF. The MUX was powered off grid using an AC to DC power adapter, and configured to store PIT codes with corresponding date/time stamps in the reader's buffer. Site checkups, including manual tuning and buffer downloads, were conducted on a semi-weekly to monthly basis.

Figure 6. PIT antenna inside the hatchery fish ladder on Eagle Creek.

***Lower Columbia River PIT Tag Detection Sites*–** In addition to the stationary PIT antennas on Eagle Creek, trawl surveys in the lower Columbia River by NOAA Fisheries in April-June, 2011-13 (Ledgerwood et al. 2004), and bird colony surveys on Sand Island October-January, 2011-14 (Collis et al. 2001), were also used as detection sites for our PIT tag data. These data were used for survival analyses. Data were obtained from PTAGIS.

Data Analysis

Juvenile Age Classification- Scale samples were gently scraped off of a gum-card with a dull scalpel, pressed between two microscope slides, secured with adhesive tape and inserted into a properly labeled (e.g., species, stream, length) coin envelope. Scales were viewed through a Microfiche reader (47X magnification) without knowledge of the fish's length or weight. Steelhead ages were determined via counting scale annuli (Davis and Light 1985). For example, if two annuli were counted on a scale, the fish was presumed to be two years old. Occasionally, rainbow trout do not lay down an annulus during their first year of life (Minard and Dye 1997). To address this potential problem, circuli between the focus and first annulus were counted in thirty-eight samples; a range of seven to twelve circuli was determined. Indistinguishable annuli between the seventh and twelfth circuli were assumed to be present. Unreadable scales (i.e., damaged or regenerated) were not incorporated into the age analysis.

Juvenile Abundance by Age Class- Using the mark-recapture data, the Chapman modification for the Lincoln-Peterson estimate was used to estimate relative abundance of winter steelhead by age class for each sample year, using the following equation:

$$N = \frac{(M+1)(C+1)}{(R+1)} - 1$$

where "N" is the population estimate, "M" is the number of marked fish in period 1 (July to early August), "C" is the total number of fish recaptured (tagged and non-tagged) in period 2 (mid-August to early September), and "R" is the number of recaptured fish in period 2 with a PIT tag. Confidence intervals were calculated from the variance of N (population estimate).

Catch Per Unit Effort (CPUE) by Age Class- CPUE was used as another method to compare relative juvenile fish abundance and was calculated as the number of juvenile steelhead caught in a stream reach divided by the effort (minutes of electrofishing). A two-way ANOVA was performed to compare CPUE data for each age class by stream and year.

Juvenile Fish Growth- Growth data for juvenile steelhead was obtained from fish that were tagged and subsequently recaptured. Growth rate was calculated as the difference in fork length between time at capture and recapture. Differences in growth rate for fish in Eagle and North Fork Eagle Creek were tested using a two-sample Mann-Whitney test.

Migration Behavior and Relative Survival to the Lower Columbia River- PIT tag detections of fish at stationary antennas within Eagle Creek and at detections sites in the lower Columbia River were downloaded and summarized by month and year to describe the migration behavior of study groups. The proportion of juveniles observed in the lower Columbia River trawl survey (and Sand Island detections) to total juveniles PIT tagged in the three study sites (upper Eagle Creek, North Fork Eagle Creek, and the hatchery) were analyzed by chi square contingency tables to compare relative survival of juvenile fish from Eagle Creek to the lower Columbia River.

PIT Tag Detection History, Probabilities and Estimated Juvenile Survival- We applied the Cormack-Jolly-Seber (CJS) model implemented within the software program MARK to estimate summer survival, between the July to early August marking event (Period 1) and the mid-August to September recapture event (Period 2), for juvenile steelhead in upper Eagle Creek and North Fork Eagle Creek. The CJS model uses data on the number of fish that were and were not detected on multiple sampling occasions to estimate the apparent survival rate (φ) and the probability of detection (ρ) on each sampling occasion (Figure 7). The July to early August tagging, mid-August to September recapture sampling (approximately one month after initial tagging), and subsequent detection data allow for a three-digit, binary, tabulation of the data that is termed a "detection history". Each fish is coded with a one to represent detection, and a zero to represent non-detection, during a sampling period. With three sampling periods for this study (July to early August tagging, mid-August to September sampling, and all subsequent detections), the CJS model can estimate the apparent survival rate from the July-August tagging until the August-September sampling period (φ), the probability of sampling fish that are alive during the August-September sampling (ρ), and the joint probability of survival and detection for all subsequent detections (λ).

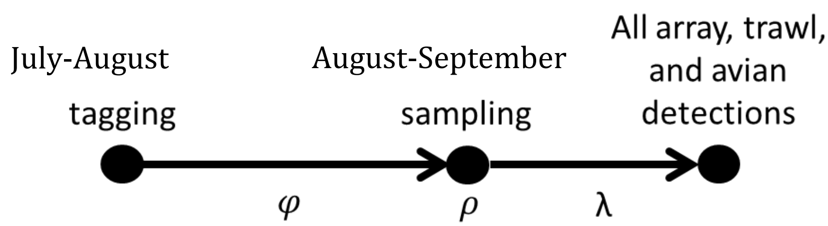

Figure 7. Cormack-Jolly-Seber model parameters for apparent survival from Period 1 (July-August tagging) to Period 2 (August-September) recapture sampling (φ), the probability of detecting fish that are alive during the August-September sampling (ρ), and the joint probability of survival and detection for all subsequent detections (λ).

Within the software program MARK, a variety of hypotheses about survival and detection probability can be evaluated using Akaike's Information Criterion (AIC), with lower AIC values indicating a better degree of fit to the data. We were interested in evaluating whether survival and detection probabilities varied by reach (upper Eagle Creek versus North Fork Eagle Creek), tagging year (2010, 2011, or 2012), or by both reach and tagging year. Alternatively, survival and/or detection probabilities may be similar or "constant" across reaches or tagging years. Each of these hypotheses was evaluated within the MARK program, using AIC to determine which hypothesis resulted in the best fit to the data. To evaluate whether length-at-tagging influenced survival, we included length as an individual covariate for modeling survival, and examined the AIC scores to determine whether length improved the degree of model fit.

Juvenile to Adult Survival- Fish detected at the lower ladder Eagle Creek antenna site two or more years after tagging (through spring of 2015) were presumed to be returning adult fish. Percent adult return was estimated from juvenile tagging to recovery, by age at return. Adult return estimates should be considered minimum estimates because adult fish may have been able to traverse the lower falls without swimming up the fish ladder during some high streamflow conditions. Differences in observed adult detections at the lower ladder for fish PIT tagged in upper Eagle Creek, North Fork Eagle Creek, and at the hatchery were analyzed by chi-square contingency table.

Genetics- Genetic samples from juvenile winter steelhead were collected in order to estimate the contribution of hatchery and wild steelhead to natural production in each of the study sites. Samples were collected in conjunction with sampling and PIT tagging in Eagle and North Fork Eagle Creeks. A small, 3 mm fin tissue sample was taken from100 natural origin winter steelhead in each creek, each year (2010-2012). Fin clips were placed in uniquely numbered vials containing 100% ethanol and provided to the U.S. Fish and Wildlife Service, Abernathy Fish Technology Center for genetic analysis (Appendix A).

Results

Marking- The total number of winter steelhead PIT tagged in upper Eagle Creek, including fish tagged during the recapture events, was 1,531 in 2010, 1,235 in 2011, and 920 in 2012. The total number of PIT tagged winter steelhead in North Fork Eagle Creek, including fish tagged during the recapture events, was 1,385 in 2010, 1,303 in 2011, and 1,087 in 2012 (Table 1). The number of residualized hatchery steelhead captured each summer in upper Eagle Creek and subsequently inserted with PIT tags was 364 in 2010, 30 in 2011, and 41 in 2012. At the hatchery, 1500 yearling hatchery winter steelhead were PIT tagged and released in April 2011 and 2012. While targeting our catch for juvenile winter steelhead, other fish were also present and incidentally captured, including cutthroat trout (*O. clarki*), coho salmon, and lamprey (*Entosphenus tridentatus and Lampetra sp.*). Two juvenile and one adult spring Chinook salmon were also caught in upper Eagle Creek (Table 2).

Table 1. Total number of juvenile winter steelhead PIT tagged and released in upper Eagle and North Fork Eagle Creeks (NFEC), 2010-2012 during both the initial sampling event and subsequent recapture events. The number of hatchery winter steelhead tagged at the hatchery as well as those hatchery residuals captured in upper Eagle Creek and PIT tagged is also included. Mortalities (Morts) are the total number of mortalities during sampling. PIT tagged mortalities are not included in the total number of PIT tagged fish. Three of the steelhead mortalities were directly related to PIT tagging while the remaining mortalities were likely due to electrofishing or handling stress.

Stream	Year					
	2010		2011		2012	
	# PIT tagged	# Morts	# PIT tagged	# Morts	# PIT tagged	# Morts
Upper Eagle Cr.	1531	24	1235	16	920	18
NFEC	1385	9	1303	8	1087	5
Upper Eagle Cr. (Hatchery Residuals)	364	0	30	0	41	0
Hatchery			1500		1500	

Table 2. Number of fish, excluding winter steelhead, incidentally caught in upper Eagle (UEC) and North Fork Eagle Creeks (NFEC), 2010-2012.

	2010		2011		2012	
	UEC	NFEC	UEC	NFEC	UEC	NFEC
Coho salmon	21	57	10	110	104	118
Cutthroat trout	78	335	26	266	75	169
Spr. Chinook salmon	3	0	0	0	0	0
Lamprey	0	9	0	6	1	5

Juvenile Age Classification- Scale samples collected in 2010 (34 fish in Eagle Creek and 66 fish from North Fork Eagle Creek) were used for age verification. Fork length (FL) of winter steelhead used in the age analysis ranged from 61 to 231 mm FL and the mean length was 199 mm FL. Based on our scale analysis, we classified fish as age-0 when they were less than or equal to 100 mm FL, classified as age-1 when between 101 mm and 150 mm FL, and classified as age-2 when greater than 150 mm FL. One fish was >230 mm FL and was classified as age-3 (Figure 8). The presence of large, naturally produced age-3 winter steelhead (or resident *O. mykiss*) was rare; their catch and abundance was not estimated further. The length frequency distribution of PIT tagged winter steelhead is shown in Figure 9 (upper Eagle Creek) and Figure 10 (North Fork Eagle Creek).

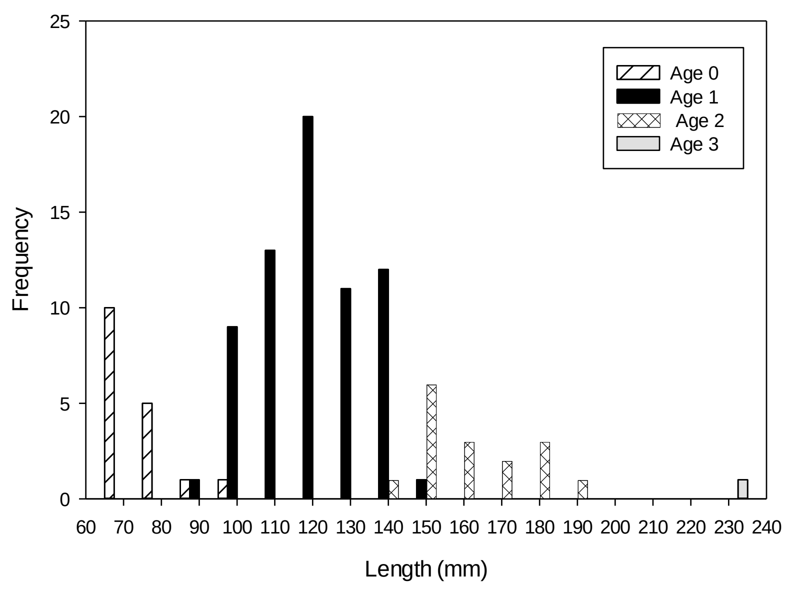

Figure 8. Fork length frequency distribution of fish used for age classification of winter steelhead in upper Eagle Creek and North Fork Eagle Creek (n=100).

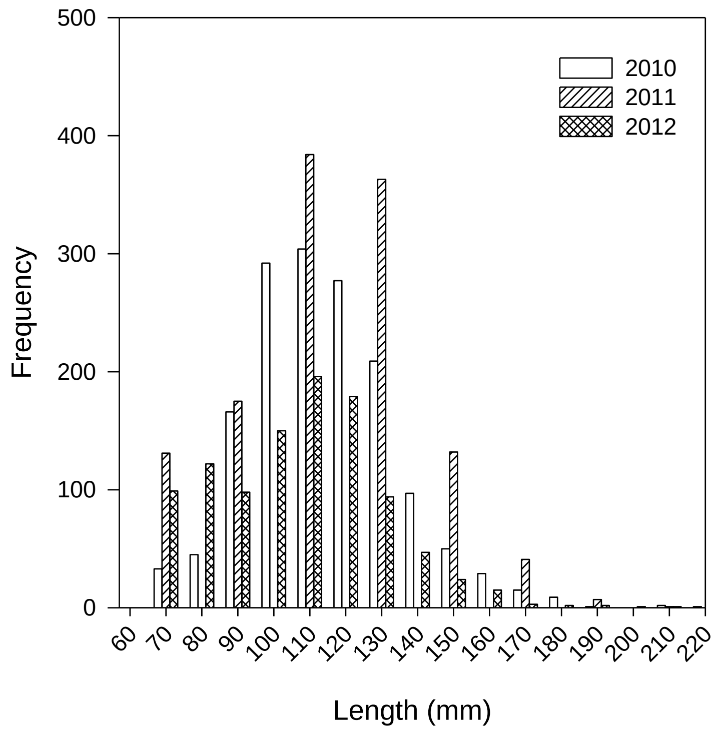

Figure 9. Length frequency distribution of PIT tagged winter steelhead in Eagle Creek, 2010-2012.

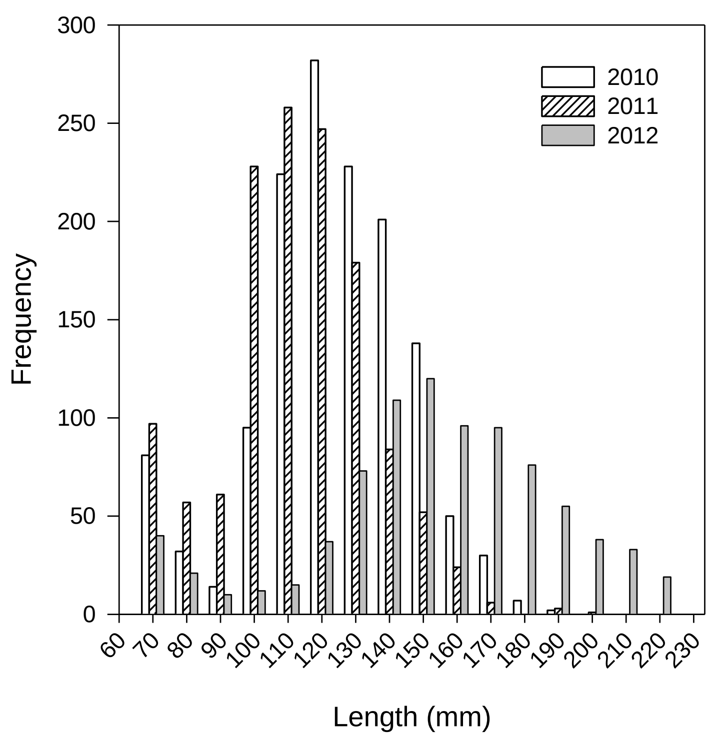

Figure 10. Length Frequency distribution of PIT tagged winter steelhead in North Fork Eagle Creek, 2010-2012.

Juvenile Abundance by Age Class-

Mark recapture data and abundance estimates are shown in Table 3. In 2010, relative abundance of winter steelhead in upper Eagle Creek was 6,203 (± 3,362 95% CI) age-0, 8,372 (± 1,562 95% CI) age-1, and 560 (± 259 95% CI) age-2 fish. Relative abundance of fish in North Fork Eagle Creek was 1,201 (± 767 95% CI) age-0, 4,896 (± 1,094 95% CI) age-1, and 245 (± 116 95% CI) age-2 fish. The estimated abundance of hatchery fish in upper Eagle Creek was 8 (± 8 95% CI) age-0, 1,959 (± 765 95% CI) age-1, and 319 (± 201 95% CI) age-2 fish. No juvenile hatchery fish were caught in North Fork Eagle Creek.

In 2011, relative abundance of winter steelhead in upper Eagle Creek was 9,051 (± 10,059 95% CI) age-0, 5,865 (± 1,883 95% CI) age-1, and 223 (± 98 95% CI) age-2 fish. Relative abundance of fish in North Fork Eagle Creek was 2,216 (± 1,447 95% CI) age-0, 4,247 (± 994 95% CI) age-1, and 210 (± 96 95% CI) age-2 fish. The estimated abundance of hatchery fish in upper Eagle Creek was 50 (± 56 95% CI) age-1 and 134 (± 170 95% CI) age-2 fish. No age-0 hatchery steelhead were caught in upper Eagle Creek in 2011. No juvenile hatchery fish were caught in North Fork Eagle Creek.

In 2012, relative abundance of winter steelhead in upper Eagle Creek was 5,580 (± 4,327 95% CI) age-0, 4,303 (± 1,522 95% CI) age-1, and 139 (± 80 95% CI) age-2 fish. Relative abundance of fish in North Fork Eagle Creek was 1,539 (± 1,678 95% CI) age-0, 4,584 (± 1,527 95% CI) age-1, and 215 (± 113 95% CI) age-2 fish. The estimated abundance of hatchery fish in upper Eagle Creek was 214 (± 184 95% CI) age-2 fish. No age-0 or age-1 hatchery steelhead were caught in upper Eagle Creek in 2012. No juvenile hatchery fish were caught in North Fork Eagle Creek.

Table 3. Mark-recapture data collected during electrofishing in upper Eagle Creek (UEC) and North Fork Eagle Creek (NFEC), 2010-12 by age/size class.
Fish were marked (M) with PIT tags in July to early August of period 1, caught (C) in mid-August to early-September of period 2 (including tagged and untagged fish) , with PIT tags recaptured (R) in period 2.
The number of hatchery winter steelhead (HWST) that were present in upper Eagle Creek during periods 1 and 2 are also recorded.
The population estimate was obtained using the Lincoln-Peterson Model for closed populations, with 95% Confidence Intervals using the Chapman method.

Age 0 between 75-100mm							Age 0 between 75-100mm							Age 0 between 75-100mm						
2010	**Stream**	**Marked (M)**	**Caught(C)**	**Recap (R)**	**Est (N)**	**+/- 95%**	**2011**	**Stream**	**Marked (M)**	**Caught(C)**	**Recap (R)**	**Est (N)**	**+/- 95%**	**2012**	**Stream**	**Marked (M)**	**Caught(C)**	**Recap (R)**	**Est (N)**	**+/- 95%**
	UEC	281	241	10	6,203	3,362		UEC	70	254	1	9,052	10,059		UEC	127	217	4	5,580	4,327
	NFEC	121	68	6	1,202	767		NFEC	96	159	6	2,216	1,447		NFEC	43	69	1	1,539	1,678
	HWST	2	2	0	8	8		HWST	0	0	0	0	0		HWST	1	0	0	1	0

Age 1 between 101-150mm							Age 1 between 101-150mm							Age 1 between 101-150mm						
2010	**Stream**	**Marked (M)**	**Caught(C)**	**Recap (R)**	**Est (N)**	**+/- 95%**	**2011**	**Stream**	**Marked (M)**	**Caught(C)**	**Recap (R)**	**Est (N)**	**+/- 95%**	**2012**	**Stream**	**Marked (M)**	**Caught(C)**	**Recap (R)**	**Est (N)**	**+/- 95%**
	UEC	1148	633	86	8,372	1,562		UEC	484	374	30	5,866	1,884		UEC	440	243	24	4,303	1,522
	NFEC	1066	256	55	4,896	1,094		NFEC	707	317	52	4,247	995		NFEC	534	239	27	4,585	1,527
	HWST	297	124	18	1,960	766		HWST	16	2	0	50	56		HWST	1	0	0	1	0

Age 2 >150mm							Age 2 >150mm							Age 2 >150mm						
2010	**Stream**	**Marked (M)**	**Caught(C)**	**Recap (R)**	**Est (N)**	**+/- 95%**	**2011**	**Stream**	**Marked (M)**	**Caught(C)**	**Recap (R)**	**Est (N)**	**+/- 95%**	**2012**	**Stream**	**Marked (M)**	**Caught(C)**	**Recap (R)**	**Est (N)**	**+/- 95%**
	UEC	101	65	11	560	259		UEC	43	55	10	223	98		UEC	39	20	5	139	81
	NFEC	84	25	8	245	116		NFEC	63	32	9	210	96		NFEC	47	35	7	215	114
	HWST	63	29	5	319	202		HWST	14	8	0	134	170		HWST	37	16	2	214	184

Note that hatchery winter steelhead (HWST) in Table 3 were grouped by fork length. Since scales were not taken from the HWST residualizing in the stream, age classification was unknown. It was possible that there were multiple age classes of HWST present in the stream, but likely they were predominately age-1 yearling fish from the spring-time hatchery release.

CPUE of Winter Steelhead by Age Class- Two way ANOVA indicated significant stream by year interactions on CPUE (fish/minute) of age-0 fish (F= 7.50, df = 2, P= <0.001) and age-1 fish (F= 3.66, df = 2, P= 0.027). Pairwise comparison tests identified statistical differences in CPUE between upper Eagle Creek and North Fork Eagle Creek for age-0 fish in 2010 and 2012 (P < 0.05), and for age-1 fish in 2010 (P < 0.05), with upper Eagle Creek having higher CPUE (Figure 11). Average CPUE for age-2 fish across all years and sampling sites was low and ranged from < 0.1 to 0.2.

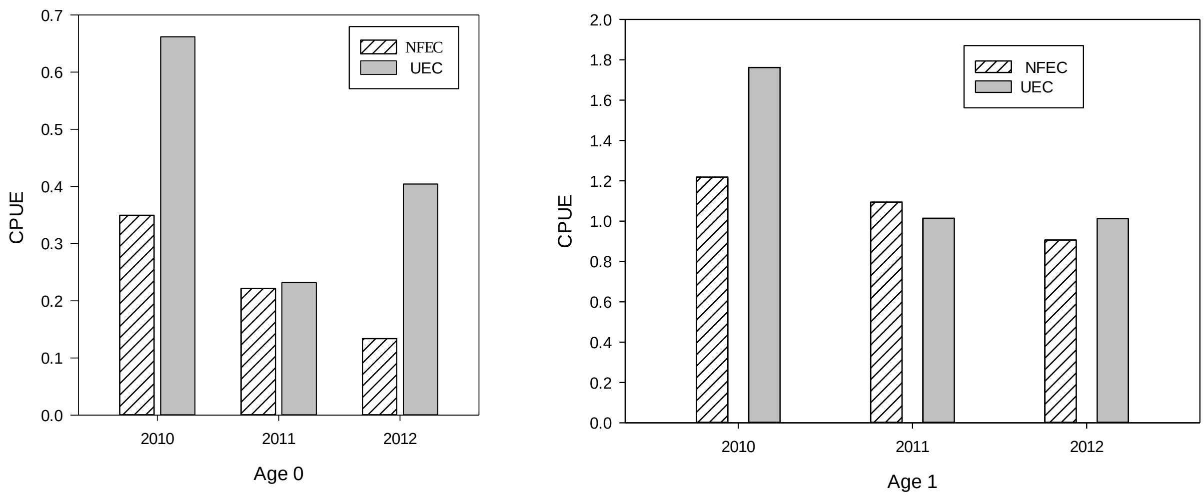

Figure 11. CPUE (fish/minute) of age-0 and age-1 winter steelhead captured in upper Eagle Creek (UEC) and North Fork Eagle Creek (NFEC) during electrofishing, 2010-12. Pairwise comparison tests suggest statistical differences in CPUE between upper Eagle Creek and North Fork Eagle Creek for age-0 fish in 2010 and 2012 (P < 0.05), and for age-1 fish in 2010 (P < 0.05).

Growth- The majority of winter steelhead recaptured for growth measurements were in the age-1 size class (Table 3). To compare growth rates between sample areas, growth data was pooled for all size classes and sample years. There was a significant difference in growth rates for juvenile steelhead in upper Eagle (n= 93) and North Fork Eagle Creeks (n = 106), with upper Eagle Creek steelhead having significantly higher growth per day ($P < 0.001$). Median growth for fish in upper Eagle Creek was 0.17 mm per day, while median growth for fish in North Fork Eagle Creek was 0.09 mm per day (Figure 12).

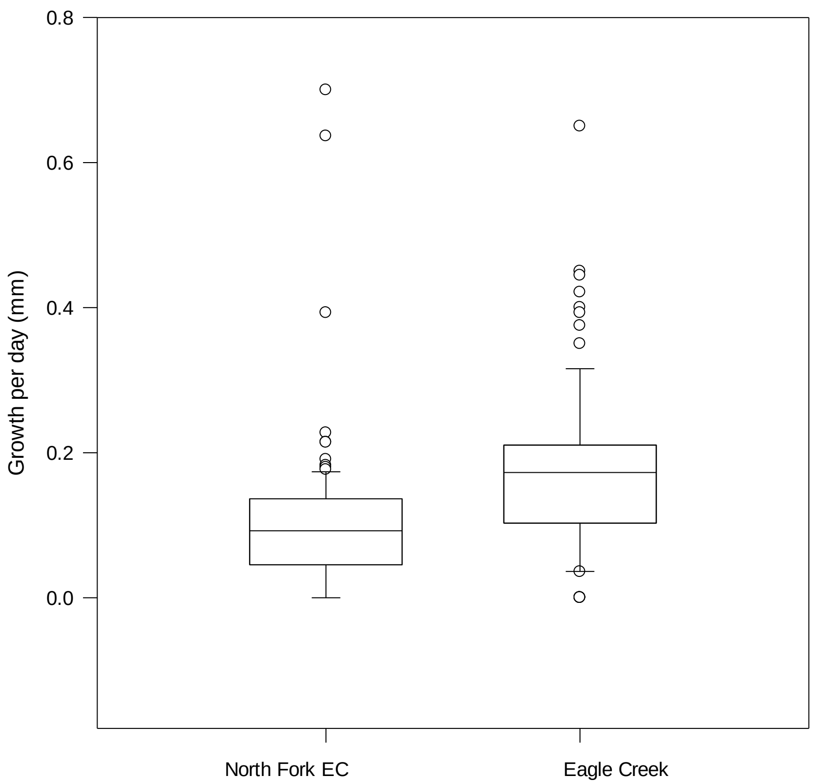

Figure 12. Average growth per day of PIT tagged juvenile winter steelhead in upper Eagle Creek (n=93) and North Fork Eagle Creek (n=106).

PIT Antenna Operations in Eagle Creek- The PIT array site near the mouth of Eagle Creek was logging consistent, continuous, and quantifiable data from July 2010 to January 2011 and between March and November 2011. Due to continual flood damage, the detection site at the mouth was decommissioned in late November 2011.

The lower fish ladder antenna was installed in December, 2011 and the hatchery ladder antenna was installed in January, 2012. The lower ladder antenna worked continuously through spring 2015, with the exception of a two week period in October 2013 during the Federal government shut down. The batteries that power the antenna were not recharged and subsequently died during this time period. In 2012, the hatchery antenna experienced periods of detection failure in all months except July and September through November. In 2013, the hatchery antenna was not operational from June through December. The hatchery operates an electric weir approximately 100 feet from the antenna site and noise interference from the weir reduced the detection efficiency of the antenna. Both the lower ladder and hatchery antennae were removed in June 2015.

PIT Tagged Fish Detections and Migration Behavior in Eagle Creek- The number of detections from out-migrating juveniles and returning adult winter steelhead varied by antenna site and month. All detections recorded at the mouth antenna were from outmigrating juvenile fish (Figure 13).

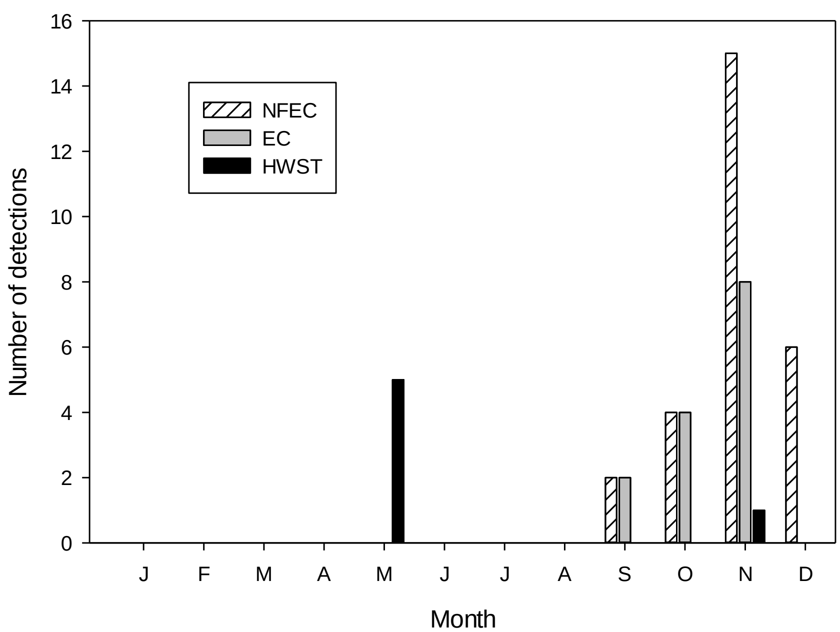

Figure 13. Monthly detections of PIT tagged juvenile winter steelhead from North Fork Eagle Creek (NFEC), upper Eagle Creek (EC), and the hatchery (HWST). Detections are at the mouth antennae in lower Eagle Creek operated from September 2010 to November 2011. The antennae was non-functional during January and February.

Twenty-seven juvenile winter steelhead tagged in North Fork Eagle Creek, 14 tagged in upper Eagle Creek, and 6 hatchery steelhead tagged at Eagle Creek NFH were detected at the mouth antenna between September 2010 and November 2011. Monthly detections of juvenile winter steelhead at the mouth site were highest in November for fish tagged in upper Eagle and North Fork Eagle Creeks. Hatchery steelhead tagged and released from Eagle Creek NFH in April 2011, were detected at the mouth in May 2011 (five fish) and November 2011 (one fish).

Outmigrating juvenile and returning adult winter steelhead were detected at the lower ladder antenna site**.** For all sample years, outmigrating juvenile winter steelhead from Eagle and North Fork Eagle Creeks were detected at the lower ladder in the Fall (September and October) of the year they were tagged or in the Spring (January, April, May, June) of the following year (Figure 14). Seven fish from upper Eagle Creek, seven fish from North Fork Eagle Creek and five hatchery fish from Eagle Creek NFH were detected as outmigrating juveniles. Hatchery steelhead were detected 2-4 weeks following release from Eagle Creek NFH.

Detections at the lower ladder antenna of returning adult fish tagged in Eagle and North Fork Eagle Creeks occurred from January through April with March having the highest number of detections for both streams (Figure 15). Adult hatchery steelhead were detected from January through March with January having the highest number of detections. Two Chinook salmon and two adult winter steelhead, tagged in a separate study at the Willamette Falls fish ladder, and one juvenile coho salmon tagged in Tryon Creek were also detected at the lower fish ladder.

Eighty-seven naturally produced winter steelhead tagged in upper Eagle Creek were subsequently detected at the hatchery ladder antenna. Eleven detections were from returning adult fish and 76 detections were from juvenile fish. Although adult fish from upper Eagle Creek were detected at the hatchery antenna, none were detected during broodstock collection at the hatchery. Juvenile fish detections were recorded 1 to 10 months after tagging. Fifteen returning adult hatchery steelhead were detected at the hatchery ladder. Fish tagged in North Fork Eagle Creek were not detected at the hatchery ladder.

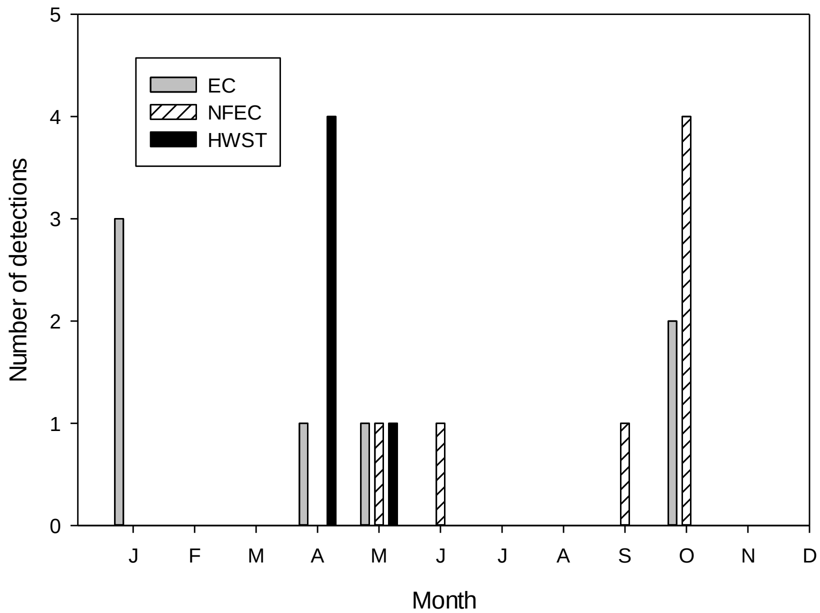

Figure 14. Monthly detections of PIT tagged juvenile winter steelhead from upper Eagle Creek (EC), North Fork Eagle Creek (NFEC), and the hatchery (HWST) as detected during downstream migration through the lower ladder in Eagle Creek, December 2011 to June 2014.

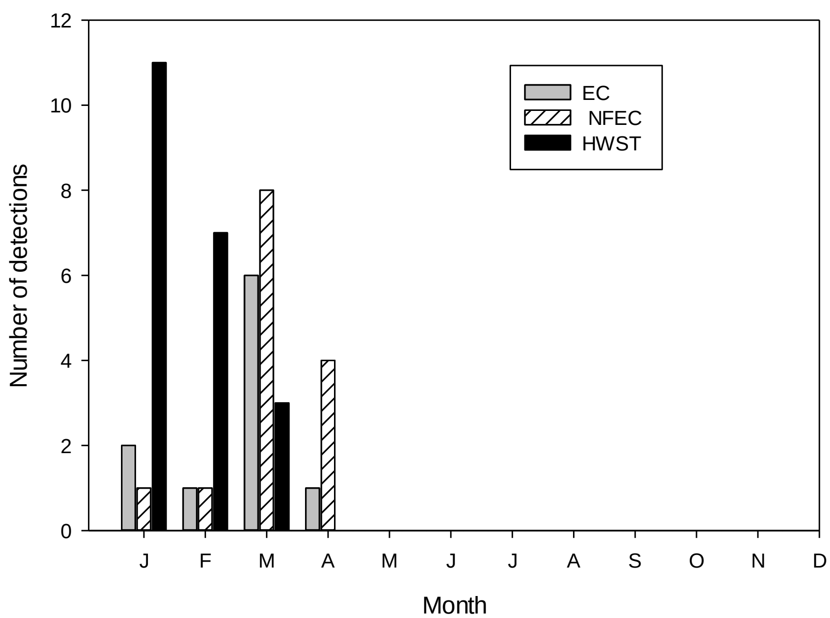

Figure 15. Monthly detections of PIT tagged adult winter steelhead at the lower ladder in Eagle Creek, 2012-15. Fish were PIT tagged as juveniles in upper Eagle Creek (EC), North Fork Eagle Creek (NFEC), and at the hatchery (HWST), 2010-12.

PIT Tagged Fish Detections, Migration, and Relative Survival to the Lower Columbia River- Juvenile winter steelhead from Eagle Creek were detected alive in the lower Columbia River, April through June. The number of fish detected in the trawl survey between 2011 and 2013 were 86 from fish tagged in upper Eagle Creek, 49 from fish tagged in North Fork Eagle Creek, and 349 from hatchery winter steelhead tagged at Eagle Creek NFH (Figure 16). Significant differences were found between the proportion of juveniles observed in the trawl survey to total juveniles PIT tagged in the three study sites ($P<0.001$). Significantly more juveniles from the hatchery releases were detected in the trawl survey. Furthermore, significantly more juveniles from upper Eagle Creek compared to North Fork Eagle Creek were detected in the trawl survey ($P=0.001$).

Juvenile mortalities reported through the East Sand Island avian surveys (October-January) were highest for hatchery fish (146), while mortalities for fish tagged in Eagle Creek (46) and North Fork Eagle Creek (47) were similar. Significantly more hatchery fish were detected ($P<0.001$).

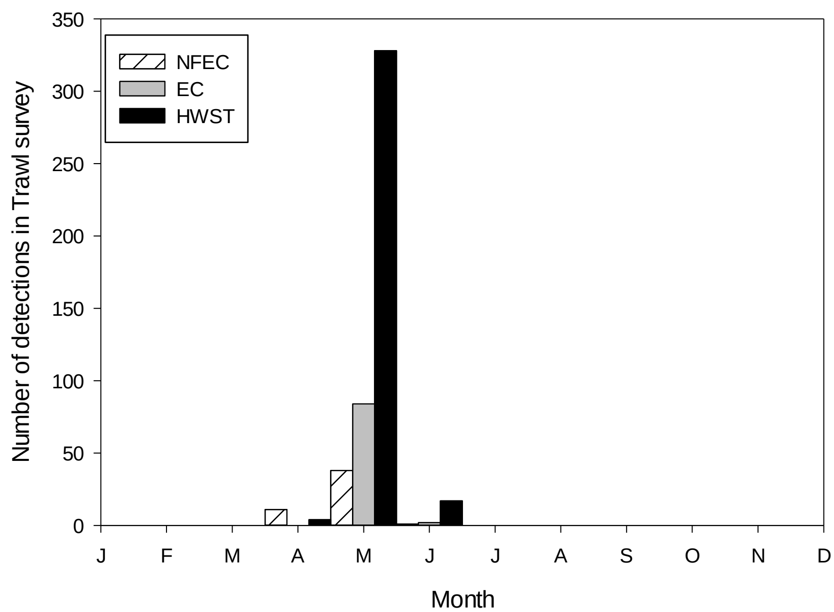

Figure 16. Monthly detections of PIT tagged juvenile winter steelhead in the *NOAA Fisheries* lower Columbia River trawl survey, 2011-13. Fish were PIT tagged as juveniles in upper Eagle Creek (EC), North Fork Eagle Creek (NFEC), and at the hatchery (HWST), 2010-12.

PIT Tag Detection History, Probabilities, and Estimated Juvenile Survival- Between 593 and 1,531 winter steelhead were tagged and released into North Fork Eagle Creek and Upper Eagle Creek, during sampling period one, July to early August, each year (Table 4). Following release, tagged fish could subsequently be detected during sampling period two (mid-August through September), or detected in the NOAA estuary PIT-trawl, in the East Sand Island avian detection surveys, or as a returning adult within one of the Eagle Creek ladders. These three periods (i.e., sampling period one, sampling period two, and the combined trawl, avian, and adult detections) allow for tabulations of “detection histories” for each tagged fish, with a “1” representing detection and a “0” representing non-detection. As is common for most mark-recapture studies, the majority of individuals were never detected again (i.e., having a "100" detection history). However, a sufficient number of fish were detected on the second and third sampling occasions to allow for estimation of apparent survival between sampling period one (July to early August) and sampling period two (mid-August through September), as well as detection probabilities during sampling period two. We use the term "summer survival" to represent apparent survival probability between sampling periods one and two.

Table 4. Detection histories for wild winter steelhead tagged and released into North Fork Eagle Creek (NFEC) and Upper Eagle Creek (UEC), 2010-2012.

	2010		2011		2012	
History	NFEC	UEC	NFEC	UEC	NFEC	UEC
100	1259	1400	788	528	575	534
110	70	102	52	34	28	27
101	55	27	22	34	11	29
111	2	2	4	1	0	3
Total	1386	1531	866	597	614	593

A total of eleven alternative model structures were evaluated (Table 5). Each model represented an alternative hypothesis on whether there was variability in summer survival or detection probabilities across years or reaches, or whether the data indicated that summer survival or detection probabilities were relatively similar or "constant" across years and reaches. Based on Akaike's Information Criterion corrected for small sample sizes (AICc), the best fitting model without length as a covariate was one that assumed that both summer survival and detection probabilities were constant across years and reaches. The estimated summer survival probability for this model was 0.90 with a profile-likelihood confidence interval of (0.56, 1.00). The estimated detection probability for the second sampling period (mid-August through September) was 0.06 with a profile-likelihood confidence interval of (0.05, 0.11). Including length at tagging as an individual covariate for summer survival resulted in an improved model fit, reducing the AICc by 3.4 units.

Based on a likelihood ratio test, the constant survival and detection model with length significantly improved the model fit compared to the same model without length ($P = 0.02$). As hypothesized, increasing length at tagging was associated with higher summer survival, with predicted summer survival increasing from 50% to 70% for 70mm to100mm (age-0) individuals, 70% to 90% survival for 101mm to150mm (age-1) individuals, and greater than 90% survival for >150mm (age-2 and older) individuals (Figure 8: Age Classification and Figure 17: Predicted Survival).

Table 5. Alternative model structures with apparent survival (Phi) and detection probability (p) either constant or allowed to vary by reach (upper Eagle Creek vs. North Fork Eagle Creek), year (2010, 2011, and 2012), or both reach and year, along with associated Akaike's Information Criterion for small sample sizes (AICc), AICc differences, number of model parameters, and deviance.

Model	AICc	Delta AICc	Num. Par	Deviance
Phi(constant) p(constant) w/length	4114.3	0.0	9	4096.2
Phi(constant) p(constant)	4117.7	3.4	8	4101.7
Phi(reach) p(constant)	4118.2	3.9	9	4100.2
Phi(constant) p(reach)	4118.2	3.9	9	4100.2
Phi(year) p(constant)	4118.5	4.2	10	4098.4
Phi(constant) p(year)	4118.7	4.4	10	4098.6
Phi(year) p(reach)	4118.9	4.6	11	4096.9
Phi(reach) p(year)	4119.0	4.8	11	4097.0
Phi(reach) p(reach)	4120.2	5.9	10	4100.2
Phi(year) p(year)	4122.8	8.5	12	4098.8
Phi (reach*year) p(reach*year)	4127.5	13.2	18	4091.4

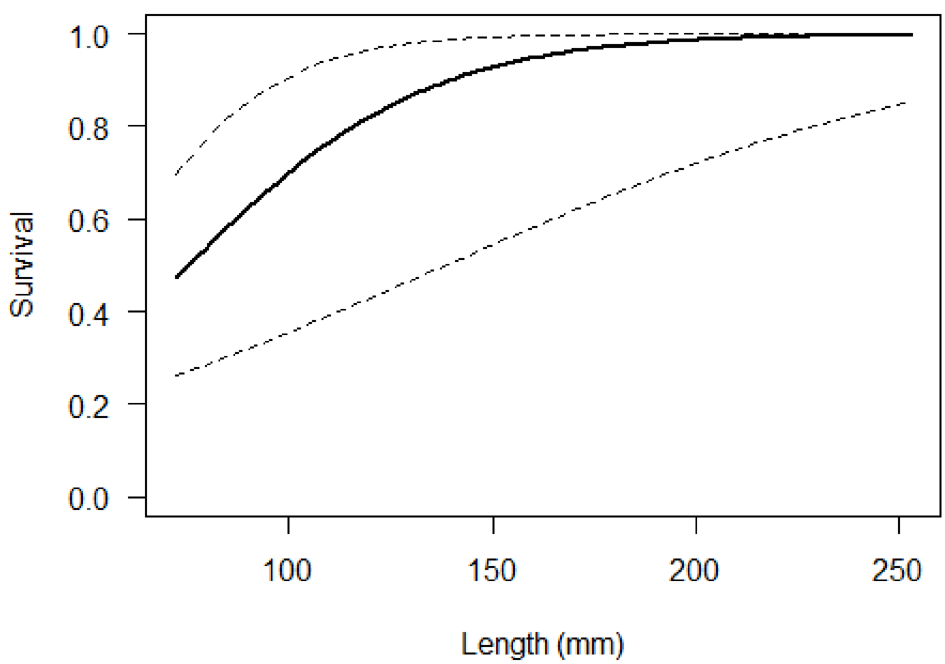

Figure 17. Predicted summer survival between sampling period one (July to early August) and sampling period two (mid-August through September) as a function of fork length at tagging for Eagle Creek winter steelhead, 2010-2012. Dotted lines represented 95% Confidence Intervals. Age-0 fish were determined to be < 101mm, age-1 fish were between 101mm and 150mm, and age-2 fish were >150mm (Figure 8).

Length-at-Tagging in Eagle Creek - We analyzed the July to early August samples from period one to determine whether there were differences in length-at-tagging between reaches and years. Four models were considered (Table 6), allowing for potential reach, year, and interactive effects (i.e., reach*year). Based on AIC, the full model with reach, year, and the reach*year interaction was identified as the best-fit model.

Table 6. Akaike's Information Criterion (AIC) values and AIC differences for models of July-early August length at tagging as a function of reach (North Fork Eagle Creek vs. Upper Eagle Creek), year (2010, 2011, and 2012), and an interaction between reach and year (Reach*Year).

Model	AIC	delta AIC
Reach + Year + Reach*Year	49309.2	0
Reach + Year	49324.5	15.3
Reach	49325.3	16.1
Year	49382.8	73.6

The parameter estimates from this model estimated that juvenile winter steelhead in North Fork Eagle Creek were significantly larger than juvenile winter steelhead in upper Eagle Creek ($P <$ 0.0001) [Figure 18]. Juveniles in North Fork Eagle Creek were estimated to be 4.5 mm longer on average than juveniles in upper Eagle Creek. However, the differences in juvenile length varied by year, with juveniles having somewhat similar lengths in 2011, but were significantly different in 2010 and 2012. Differences in length at tagging can largely be explained by examining age at tagging (Table 3). In 2010 18% of the tagged fish were age-0 in upper Eagle Creek compared to 9% in North Fork Eagle Creek; 11% were age-0 in both streams in 2011; and 20% were age-0 fish in upper Eagle Creek compared to 7% in North Fork Eagle Creek in 2012.

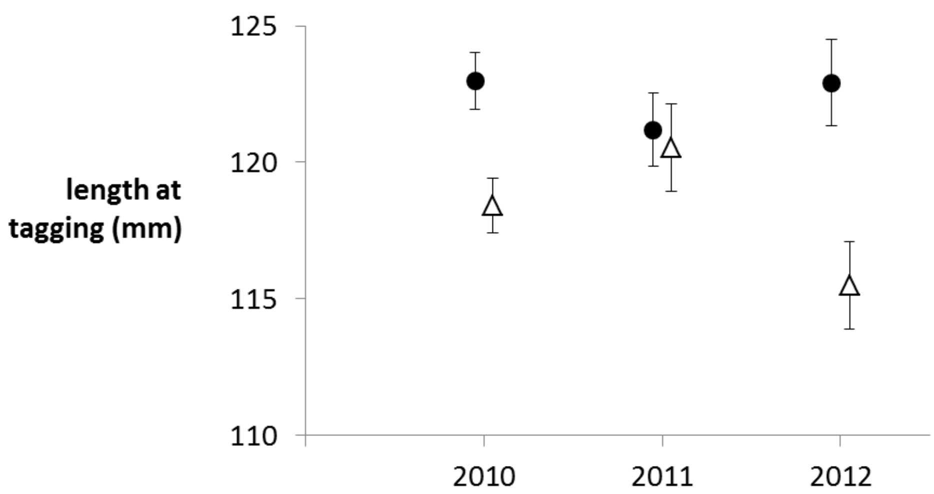

Figure 18. Model estimates for mean July to early August fork length at PIT tagging (mm) for North Fork Eagle Creek (filled circles) and upper Eagle Creek (open triangles) juvenile winter steelhead, 2010-2012. Error bars represent 95% confidence intervals.

Juvenile-to-Adult Survival- The number of adult detections of winter steelhead at the lower ladder antenna for fish tagged in upper Eagle Creek, North Fork Eagle Creek, and at Eagle Creek NFH was 11, 15, and 21, respectively. The differences in adults observed between study sites were not significant (P= 0.12). Age class structure of returning adults ranged from 3-5 years old, with juvenile-to-adult survival ranging from 0.3% to 0.6% (Table 7). These are conservative survival estimates (i.e., they likely underestimate survival) since an unknown number may have passed through the lower ladder during high flow conditions.

Table 7. Observed age class structure of PIT tagged winter steelhead from upper Eagle Creek, North Fork (NF) Eagle Creek, and Eagle Creek National Fish Hatchery (NFH) returning to the lower ladder and relative percent survival from juvenile tagging (2010-12) to adult detection (2011-15).

Study Site	Age-3	Age-4	Age-5	Total Adults Observed	Total Juveniles Tagged	Relative Percent Survival
Upper Eagle Creek	6	5	0	11	3,686	0.3%
NF Eagle Creek	9	5	1	15	3,775	0.4%
Eagle Creek NFH	14	7	0	21	3,435	0.6%

Genetics- Abernathy Fish Technology Center analyzed 200 samples of juvenile winter steelhead collected in 2010 and 2011 (Appendix A). Bingham (2013) found that hatchery influence in naturally produced juvenile winter steelhead was significantly higher in upper Eagle Creek than in North Fork Eagle Creek in 2010 and 2011; however, because of the low number of loci analyzed and the low genetic divergence between the hatchery and wild baseline, it was difficult to determine the level and occurrence of introgression. The wild baseline appeared to contain evidence for hatchery introgression as well.

Discussion

While less than our initial target of tagging 4,500 juvenile winter steelhead in each stream, we successfully PIT tagged 3,686 juvenile winter steelhead in upper Eagle Creek and 3,775 juvenile winter steelhead in North Fork Eagle Creek. We constructed and maintained PIT antennas at three locations in Eagle Creek; however, the instream antenna near the mouth of Eagle Creek was blown out during two fall/winter floods and was ultimately not as effective as originally planned. This affected our ability to detect and estimate the number of downstream migrating juvenile fish and smolts. Even with this limitation, we were able to collect comparative information on fish movement, relative abundance, growth, survival, and genetics of juvenile winter steelhead from both streams.

Our estimates of population abundance of juvenile winter steelhead in upper Eagle Creek and North Fork Eagle Creek varied considerably between sample years and study sites. Overall, relative abundance of juvenile winter steelhead, in particular age-0 fish, was greater in upper Eagle Creek. The annual differences in our estimates were also influenced by sampling effort, stream conditions, and electrofishing experience of the sampling crew. Even so, it is not unusual for salmonid population numbers to fluctuate in response to habitat conditions, adult returns, and changes in abundance of other species (Lichatowich 1999). Additionally, our estimates do not consider possible recruitment that occurred between the mark and recapture sampling periods (Boughton 2010). For example, we avoided capturing and handling fish less than 75 mm. It is likely that additional fish recruited between sampling periods and their contribution was not reflected in our estimates.

Limiting our sampling effort to steelhead > 74 mm for the purpose of PIT tagging likely excluded a large percentage of fish < 75 mm from our population estimate of age-0 fish. In an earlier study in Eagle Creek during summer 2007, approximately one-third of the age-0 winter steelhead sampled were between 75 and 100 mm, with the other two-thirds of age-0 winter steelhead between 50 and 70 mm (Figure 3 of Kavanagh et al. 2009). Because of this sampling limitation for age-0 winter steelhead in 2010-12, another useful metric for examining relative abundance is the ratio of estimated abundance between upper Eagle Creek and North Fork Eagle Creek. Examining this ratio, an average of 4.3 times more age-0 winter steelhead between 75 and 100 mm were found in upper Eagle Creek during 2010-12 as compared to North Fork Eagle Creek. This ratio can be used for age-1 and age-2 fish as well. An average of 1.3 times more age-1 and age-2 fish were found in upper Eagle Creek during our sampling in 2010-12.

Based on available habitat area, one would expect more juvenile fish in upper Eagle Creek. As described in Kavanagh et al. (2009), both creeks have a similar percentage of pools, riffles and glides; however, upper Eagle Creek is on average a wider stream (14.6m) compared to North Fork Eagle Creek (7.5m). Upper Eagle Creek has approximately 178,302 m^2 total habitat area and North Fork Eagle Creek has approximately 115,664 m^2 total habitat area. Kavanagh et al. (2009) also found significantly higher rearing densities (fish/m^2) for age-0 winter steelhead (50 - 109 mm) in upper Eagle Creek, with no significant differences in rearing densities for age-1 fish (>109 mm). Utilizing the habitat area estimates from Kavanagh et al. (2009), our estimate of rearing density in 2010-12 was approximately 3 times higher in upper Eagle Creek for age-0 winter steelhead (75 - 100 mm), and similar for age-1and age-2 steelhead (> 100 mm). Comparative rearing density estimates in 2010-12 were consistent with Kavanagh et al. (2009). Also consistent with Kavanagh et al. (2009), hatchery juvenile fish were found residualizing in upper Eagle Creek but not in the North Fork Eagle Creek during 2010-12.

The estimated number of hatchery residuals found over-summering in upper Eagle Creek was much lower in 2011(184) and 2012 (216), compared to 2,287 estimated during 2010. This can partially be explained by the number of winter steelhead released from the hatchery each year. Hatchery yearling steelhead releases were 111,606 in 2010, 67,560 in 2011, and 49,000 in 2012. The current hatchery release goal is 95,000 yearling steelhead smolts.

Large numbers of hatchery juvenile steelhead that residualize in a stream can negatively affect growth rates of wild *O. mykiss* (McMichael et al. 1997). Because hatchery residual juveniles are present in upper Eagle Creek and not found in North Fork Eagle Creek, we hypothesized that juvenile winter steelhead growth rates would be negatively affected by hatchery influence in upper Eagle Creek. Our data did not support our hypothesis, with average growth per day being significantly greater for juvenile winter steelhead in upper Eagle Creek compared to North Fork Eagle Creek. A number of environmental and genetic factors can influence growth rates.

Stream temperature can influence growth rates of juvenile steelhead (Doctor et al. 2014). While temperature was not measured consistently year-round, a previous study found that the two study sites had similar summer water temperature, with North Fork Eagle Creek averaging 15.3 C and upper Eagle Creek 15.6 C (June – August 2007 data in Kavanagh et al. 2009). Another possibility is that increased hatchery introgression from naturally spawning hatchery steelhead in upper Eagle Creek resulted in faster growing juvenile fish, as was found in a study with domesticated coho salmon (Tymchuk et al. 2006) and with hatchery by wild crosses of steelhead (Reisenbichler and McIntyre 1977). Alternatively, the larger, more complex habitat area and possibly increased food availability in upper Eagle Creek may have contributed to the higher abundance and growth rates that were observed in this study (Cederholm et al. 1999; Fausch and Northcote 1992; and Nielson 1992).

Reisenbichler and McIntyre (1977) found significantly higher survival of juvenile steelhead in a stream when wild x wild crosses were mated as compared to hatchery x wild and hatchery x hatchery crosses. The hatchery influence from genetic samples collected in 2010 and 2011 was significantly higher in upper Eagle Creek than in North Fork Eagle Creek (Bingham 2013 in Appendix A). Based on those results, our hypothesis was that North Fork Eagle Creek winter steelhead would have higher over-summer survival. We did not find differences in over-summer survival of juvenile winter steelhead in upper Eagle and North Fork Eagle Creeks. It is possible that a difference in survival between the two streams did not exist; however, the statistical power in our study, which is a function of the number of fish that were tagged and later detected, may have been too low to detect differences in survival if they did exist. In addition, the time period that we estimated over-summer survival was between July/early-August and mid-August/September, a two to six week time period which may have not been long enough for survival differences to become evident.

While we did not find differences in over-summer survival between upper Eagle Creek and North Fork Eagle Creek, we found significant differences in survival based on length, which provided an estimate of over-summer survival of the different age classes. Larger fish had a higher probability of survival. Based on length, over-summer survival of age-0 fish was estimated to be between 50% and 70%, age-1 fish survival was estimated to be between 70% to 90%, and age-2 and older fish survived at a rate greater than 90%.

The majority of juvenile winter steelhead from upper Eagle and North Fork Eagle Creeks were detected emigrating in the fall, whereas most hatchery fish were detected emigrating in the spring. It was not unexpected to detect hatchery fish in May 2011 shortly after release, but we also expected to see some wild juveniles (age-1 fish tagged in 2010) to be detected in that time period as well. Detection probability was likely low with low numbers of juvenile fish being detected as they migrated past the lower ladder and mouth antennas in Eagle Creek. Spring outmigration of age-0 and age-1 juvenile steelhead in Clackamas River tributaries, including North Fork Eagle Creek and upper Eagle Creek, has been documented in other studies (Hansen et al. 2009; Kavanagh et al. 2009) and year-round emigration of juvenile salmon and steelhead is documented from dam counts in the upper Clackamas River (Wyatt 2009). In another northwest stream, Leider et al. (1986) described the seasonal migratory behavior of parr and presmolt steelhead. They found that the movement of pre-smolt parr downstream from a smaller order creek to a larger river area was a successful survival strategy for becoming smolts in the subsequent year. The success of this migratory behavior in the Clackamas basin is unknown.

The proportion of juvenile winter steelhead from initial PIT tagging in Eagle Creek to later detection in the lower Columbia River during spring time trawl surveys (Ledgerwood et al. 2004) provided an indication of comparative survival between the hatchery release, upper Eagle Creek, and North Fork Eagle Creek. We presumed that juvenile fish sampled in the lower Columbia River were smolts and were transitioning to life in a marine environment. We found significantly higher detections in the trawl survey from the hatchery release compared to naturally produced fish from Eagle Creek. Naturally produced juveniles from upper Eagle Creek had significantly higher detections than from North Fork Eagle Creek. Hatchery fish were all tagged as yearling fish just prior to release while naturally produced fish were tagged in the summer 5-7 months prior to the trawl surveys, so the larger number of hatchery fish detected in the trawl survey was not unexpected. The higher proportional detection of fish from upper Eagle Creek suggests that those fish had higher survival from tagging to the lower Columbia River as compared to the North Fork Eagle Creek winter steelhead.

The proportional detection of PIT tags from avian predation in the lower Columbia River was significantly greater for hatchery steelhead released from Eagle Creek NFH compared to the number of natural origin juvenile winter steelhead from upper Eagle and North Fork Eagle Creeks. The higher mortality of hatchery smolts from avian predation may be due to their relative abundance, their relative vulnerability to avian predation because of their tendency towards surface orientation compared to their wild counterpart (Collis et al. 2001), or some combination of these factors.

There is evidence that higher growth rates and/or larger smolt size in freshwater can result in higher survival in the marine environment (Ward and Slaney 1988; Thompson and Beauchamp 2014). We did not find that the higher growth rates observed for upper Eagle Creek steelhead was associated with higher juvenile-to-adult return rates compared to North Fork Eagle Creek. While we estimated the growth rates of mixed age groups of juvenile steelhead (the growth rate of juvenile winter steelhead in upper Eagle Creek was greater than in North Fork Eagle Creek), the actual size during the time of smoltification was unknown. The size of fish we sampled and tagged was largely influenced by the higher relative abundance of age-0 winter steelhead collected and tagged in upper Eagle Creek as compared to North Fork Eagle Creek. We found that differences in juvenile length at tagging varied by year, with juveniles in upper Eagle Creek and North Fork Eagle Creek having similar lengths in 2011, but in 2010 and 2012 fish tagged in the North Fork Eagle Creek were significantly larger.

Because the number of winter steelhead smolts from upper Eagle Creek and North Fork Eagle Creek were not quantified in our study, we could not estimate smolt-to-adult survival. We were able to estimate the relative survival of adult fish from juvenile tagging to adult detection at the lower ladder in Eagle Creek. Since the ladder detections most likely did not detect 100% of the fish passing the falls, survival estimates should be considered conservative, and we assumed that both upper Eagle Creek and North Fork Eagle Creek had equal detection probabilities. From tagging to adult detection, upper Eagle Creek survival was estimated at 0.3% and North Fork Eagle Creek was estimated at 0.4%. These differences were not significant.

Survival of hatchery steelhead from tagging to adult detection at the lower ladder was estimated at 0.6%, and from tagging to detection at the hatchery ladder was 0.4%. While more adult hatchery fish were detected in Eagle Creek, the difference between hatchery and wild (upper Eagle and North Fork Eagle creeks combined) was not significant. At our tagging levels and at the estimated survival rates, the difference in detections between the two groups would have to be greater than 100% before statistically significant differences would be found. While not statistically significant, hatchery steelhead from Eagle Creek NFH had the highest percent survival from juvenile tagging to adult detection, which was not surprising since these fish were tagged as yearling smolts just prior to release.

The age of PIT tagged adult winter steelhead detected at the lower ladder ranged from three to five years old. Hatchery adult steelhead in Eagle Creek tend to return earlier in the season than naturally produced fish (Kavanagh et al. 2009). In our study we also observed temporal segregation in return timing of hatchery and natural origin adult winter steelhead in Eagle Creek. Although natural origin adult winter steelhead were detected in January and February at the lower ladder, peak migration of returning adults occurred in March. The peak migration for returning hatchery steelhead was in January. Although based on few fish, upper Eagle Creek and North Fork Eagle Creek had similar adult return timing.

Including genetic collections from Kavanagh et al. (2009), Bingham (2013) found that the wild, Eagle Creek genetic baseline appears to contain evidence for hatchery introgression (Appendix A: Table 1, Figure 2). Only a few of the pairwise locus comparisons were significant, suggesting that introgression from the hatchery has occurred for many generations (e.g., Allendorf et al. 2001). Bingham (2013) also concluded that the hatchery admixture in wild winter steelhead populations in the Eagle Creek basin appears dynamic. The hatchery influence from samples collected in 2007, 2010 and 2011 was significantly higher in upper Eagle Creek than in North Fork Eagle Creek; however, in 2005 the hatchery influence was significantly higher in North Fork Eagle Creek (no difference in 2006). Thus, it is important to interpret the genetic impact of the hatchery in the context of the timeframe in which it was sampled.

Based on the information collected and analyzed to date as part of this project, we cannot conclude that freshwater growth, migration behavior, or survival in upper Eagle Creek was negatively impacted by naturally spawning hatchery steelhead when compared to North Fork Eagle Creek. It is also important to note that the North Fork Eagle Creek is not a pristine, production area for wild winter steelhead, but typically does have less hatchery influence than in upper Eagle Creek. Both upper Eagle Creek and North Fork Eagle Creek naturally produce juvenile and adult salmon and steelhead. Brignon et al. (2012) also suggested that the habitat conditions in upper Eagle Creek may be best for producing age-0 winter steelhead while the habitat conditions in North Fork Eagle Creek may be better for coho salmon.

Starting with brood year 2015, the winter steelhead hatchery program was switched from a segregated, early-run stock to an integrated broodstock with the late-run winter steelhead from the Oregon Department of Fish and Wildlife's Clackamas hatchery. After this new hatchery steelhead program becomes established in a few years, it is recommended that additional evaluations occur to monitor for changes in stream ecology, including hatchery residualism, the proportion of hatchery fish spawning (pHOS) in the stream, and associated genetic influence. Berejikian et al. (2012) describe case studies that could serve as a model for evaluation of an integrated steelhead hatchery program. Starting in brood year 2013, Oregon Department of Fish and Wildlife also initiated a 240,000 spring Chinook smolt program at Eagle Creek NFH. Eagle Creek NFH also continues to rear coho salmon for release into Eagle Creek and for transfer to upper Columbia and Snake River reintroduction programs run by Tribal governments (USFWS 2007). All programs at Eagle Creek NFH should be evaluated periodically to ensure parameters in the Hatchery and Genetic Management Plans and ESA Biological Opinion are met.

While not an objective of the study, it is important to recognize the significant contributions made by students employed during summer field work through the Student Temporary Employment Program (STEP). An article documenting some of these contributions was published in the U.S. Fish and Wildlife Service Eddies Spring 2011 edition that highlighted the students' work on the Eagle Creek project (Attachment B).

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Literature Cited

Allendorf, F.W., R.F. Leary, P. Spruell, and J.K. Wenburg. 2001. The problems with hybrids: settting conservation guidelines. Trends in Ecology & Evolution 16(11):613-622.

Araki, H., W. R. Ardren, E. Olsen, B. Cooper, and M. S. Blouin. 2007. Reproductive success of captive-bred steelhead trout in the wild: Evaluation of three hatchery programs in the Hood River. Conservation Biology 21(1):181-190.

Berejikian, B.A., D.A. Larsen, P. Swanson, M.E. Moore, C.P. Tatara, W. L. Gale, C.R. Pasley, and B.R. Beckman. 2012. Development of natural growth regimes for hatchery-reared steelhead to reduce residualism, fitness loss, and negative ecological interactions. Environmental Biology of Fishes 94:29-44.

Bingham, D. 2013. Genetic analysis of juvenile steelhead (*Oncorhynchus mykiss*) samples collected in upper Eagle Creek and North Fork Eagle Creek, OR. U.S. Fish and Wildlife Service, Abernathy Fish Technology Center, Longview, WA (as provided in Appendix A).

Boughton, D.A. 2010. Estimating the size of steelhead runs by tagging and monitoring migrants. North American Journal of Fisheries Management 30:89-101.

Brignon, W.R., D.E. Olson, H.A. Schaller, and C.B. Schreck. 2012. Factors influencing density, distribution, and mesohabitat selection of juvenile wild salmonids and residual hatchery winter steelhead. Aquaculture 362-363 (2012) 137-147.

Cederholm, C.J., M.D. Kunze, T. Murota, and A. Sibatani. 1999. Pacific salmon carcasses: Essential contributions of nutrients and energy for aquatic and terrestrial ecosystems. Fisheries 24(10):6-15.

Chilcote,M.W. 2003. Relationship between natural productivity and the frequency of wild fish in mixed spawning populations of wild and hatchery steelhead (*Oncorhynchus mykiss*), Canadian Journal of Fisheries and Aquatic Sciences 60: 1057–1067.

Collis, K., D.D. Roby, D.P. Craig, B.A. Ryan, and R.D. Ledgerwood. 2001. Colonial waterbird predation on juvenile salmonids tagged with passive integrated transponders in the Columbia River esturary: Vulnerability of different salmonid species, stocks, and rearing types. Transactions of the American Fisheries Society 130:385-396.

Columbia Basin Fish and Wildlife Authority (CBFWA). 1999. PIT Tag Marking ProceduresManual. PITTag Steering Committee, Version 2.0, Portland, Oregon.

Davis, N. D. and J. T. Light. 1985. Steelhead age determination techniques. (Document submitted to annual meeting of the INPFC, Tokyo, Japan, November 1985.) 41 pp. University of Washington, Fisheries Research Institute, FRI—UW—85O6. Seattle.

Doctor, K., B. Berejikian, J.J. Hard, and D. VanDoornik. 2014. Growth mediated life history traits of steelhead reveal phenotypic divergence and plastic response to temperature. Transactions of the American Fisheries Society 143(2):317-333.

Fausch, K.D. and T.G. Northcote. 1992. Large woody debris and salmonid habitat in a small coastal British Columbia stream. Canadian Journal Fisheries and Aquatic Sciences 49:682-693.

Hansen, B., B. Strobel, and B. Wymore. 2009. Steelhead and coho salmon smolt production, length distributions, and emigration patterns in the Clackamas River Basin, 2006-2007. USDA Forest Service, Pacific Northwest Research Station and the Portland Water Bureau. *In* July 2009 Fisheries Partnerships in Action, Accomplishment Report for the Clackamas River Fisheries Working Group, 2006-2008: USDA Forest Service, U.S. Fish and Wildlife Service, Oregon Department of Fish and Wildlife, U.S. Bureau of Land Management, and Portland General Electric.

Kavanagh M., W.R. Brignon, D. Olson, S. Gutenberger, A. Matala, and W. Ardren. 2009. Ecological and Genetic Interactions between Hatchery and Wild Steelhead in Eagle Creek, Oregon. Final Report. U.S. Fish and Wildlife Service, Columbia River Fisheries Program Office, Vancouver, WA.

Ledgerwood, R.D., B.A. Ryan, E.M. Dawley, E.P. Nunnallee, and J.W. Ferguson. 2004. A surface trawl to detect migrating juvenile salmonids tagged with passive integrated transponder tags. North American Journal of Fisheries Management 24(2):440-451.

Leider, S.A., M.W. Chilcote, and J.J. Looch. 1986. Movement and survival of presmolt steelhead in a tributary and the main stem of a Washington river. North American Journal of Fisheries Management 6(4):526-531.

Lichatowich, J.A. 1999. Salmon without rivers: a history of the Pacific salmon. Island Press, Washington, D.C.

Lynch, M., and M. O'Hely. 2001. Captive breeding and the genetic fitness of natural populations. Conservation Genetics 2(4):363-37.

McMichael, G.A., C.S. Sharpe, and T.N. Pearsons. 1997. Effects of residual hatchery-reared steelhead on growth of wild rainbow trout and spring Chinook salmon. Transactions of the American Fisheries Society 126:230-239.

Minard, R.E. and J.E. Dye. 1997. Rainbow trout sampling and aging protocol. Alaska Department of Fish and Game, Special Publication No. 98-2, Anchorage.

Nielson, J.L. 1992. Microhabitat-specific foraging behavior, diet, and growth of juvenile coho salmon. Transactions of the American Fisheries Society 121(5):617-634.

Reisenbichler, R.R. and J.D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*. Journal of the Fisheries Research Board of Canada 34(1):123-128.

Thompson, J.N. and D.A. Beauchamp. 2014. Size-selective mortality of steelhead during freshwater and marine life stages related to freshwater growth in the Skagit River, Washington. Transactions of the American Fisheries Society 143:910-925.

Tymchuk, W.E., C. Biagi, R. Withler, and R.H. Devlin. 2006. Growth and behavioral consequences of introgression of a domesticated aquaculture genotype into a native strain of coho salmon. Transactions of the American Fisheries Society 135(2):442-455.

U.S. Fish and Wildlife Service (USFWS). 2007. Eagle Creek National Fish Hatchery assessments and recommendations, final report July 2007. Pacific Region, Portland, Oregon. http://www.fws.gov/pacific/fisheries/Hatcheryreview

Ward, B.R. and P.A. Slaney. 1988. Life history and smolt-to-adult survival of Keogh River steelhead trout (Salmo gairdneri) and the relationship to smolt size. Canadian Journal of Fisheries and Aquatic Sciences 45:110-1122.

Wyatt, G.J. 2009. Abundance and run timing of salmonids collected at North Fork juvenile bypass facility, 2006-2008. Portland General Electric. *In* July 2009 Fisheries Partnerships in Action, Accomplishment Report for the Clackamas River Fisheries Working Group, 2006-2008: USDA Forest Service, U.S. Fish and Wildlife Service, Oregon Department of Fish and Wildlife, U.S. Bureau of Land Management, and Portland General Electric.

Appendix A:

Bingham, D. 2013. Genetic analysis of juvenile steelhead (*Oncorhynchus mykiss*) samples collected in upper Eagle Creek and North Fork Eagle Creek, OR. U.S. Fish and Wildlife Service, Abernathy Fish Technology Center (AFTC), 1440 Abernathy Creek Road, Longview, WA 98632

Genetic analysis of juvenile steelhead (*Oncorhynchus mykiss*) samples collected in upper Eagle Creek and North Fork Eagle Creek, OR

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July 2013

Introduction

Eagle Creek is located within the Clackamas River Basin, OR, and contains a wild population of steelhead (*Oncorhynchus mykiss*) (wild referring to fish born in the natural environment regardless of ancestry). Wild steelhead in Eagle Creek are included in the lower Columbia River Evolutionarily Significant Unit (ESU) and are listed as “threatened” under the Endangered Species Act (ESA; Lower Columbia River ESU, 63 FR 13347; March 19, 1998).

Hatchery propagation of steelhead at Eagle Creek National Fish Hatchery (ECNFH) was implemented as mitigation for loss of fishery resources in the Columbia River basin. The original ECNFH winter-run broodstock was largely derived from out-of-basin Big Creek Hatchery stock from the Lower Columbia River with a smaller component of local wild stocks.

Hatchery steelhead return to spawn in Eagle Creek from December through March, whereas wild late-run steelhead return from February to June. This temporal distinction has been viewed as beneficial because it allows for a targeted fishery on early returning hatchery steelhead. Managers have assumed little hybridization occurs between wild and hatchery fish because of distinct spawning locations and differences in spawning time.

Little is known about the genetic contribution of wild and hatchery fish in the Eagle Creek system. Genetic analysis of 16 microsatellites showed that divergence between the hatchery and wild populations in Eagle Creek is small (F_{ST} = 0.018; 95% CI 0.012-0.025) (Matala et al. 2007). With such small divergence 16 microsatellites provide little power to assign individuals as hatchery, wild, or hybrid (Vaha and Primmer 2006). Nevertheless, analysis of wild juvenile steelhead from upper Eagle Creek has indicated hatchery ancestry in wild born fish, suggesting successful reproduction of hatchery fish in the wild (Kavanagh et al. 2009).

Our objective was to evaluate the resolution provided by the available genetic baseline (Matala 2007) for distinguishing hatchery from wild *O. mykiss* in Eagle Creek. We then analyzed samples of 200 juvenile steelhead sampled from upper Eagle Creek and North Fork Eagle Creek in 2010 and 2011 to determine whether accurate assignment to the wild and hatchery baselines is possible. Finally, to identify trends in the genetic data, we reexamined samples from the Eagle Creek basin that were analyzed in the Matala et al. (2007). Sampling information, laboratory analysis, and genetic summary statistics for the 15 microsatellites in the hatchery and wild genetic baselines and in samples collected prior to 2010 can be found in Matala et al. (2007).

Methods

To detect genetic patterns and obtain a multivariate analysis of the hatchery and wild baselines, we performed an individual-based principal coordinate analysis (PCoA) of a covariance-standardized genetic distance matrix in GENALEX v6.0 (Peakall and Smouse 2006).

If the wild and hatchery baselines show extensive admixture with each other they may not be appropriate for determining the ancestry of wild-born *O. mykiss*. We used the Bayesian clustering model in STRUCTURE Ver. 2.3.3 (Pritchard et al. 2000) to identify admixture between the hatchery and wild baselines. STRUCTURE gives a q_i-value for each individual, which represents the estimated proportion of an individual's genotype that was derived from the hatchery (i.e., an individual of hatchery ancestry should exhibit q_i = 1.00). We performed 10 independent runs using a burn-in period of 10,000, 50,000 batches, and the admixture and 'independent allele frequency' models. We forced the model to recognize only two populations (k = 2; i.e., hatchery and wild) and included the 163 hatchery and 100 wild fish from the baseline as priors.

We used linkage disequilibrium (i.e., nonrandom association of genotypes between loci) to gain inference on the 'age' of hybridization between hatchery and wild fish in sites that appeared to contain hatchery introgression. When genetically distinct populations interbreed, linkage will initially be high because populations will contain parental types and many early generation hybrids (Allendorf et al. 2001). In contrast, the absence of linkage disequilibrium suggests low power of detection (i.e., due to similar allele frequencies between populations or recent introgression by later-generation hybrids) or that hybridization has progressed to a 'hybrid swarm' in which all individuals are hybrids. We used exact tests in ARLEQUIN (Excoffier et al. 2005) to test for significant linkage disequilibrium between all possible pairwise locus comparisons and determined statistical significance at α=0.05.

Results and Discussion

Analysis of 15 microsatellite loci showed that the hatchery and wild genetic baseline collections of *O. mykiss* in the Eagle Creek system contain similar allele frequencies ($F_{ST} \leq 0.02$; Figure 1). The small number of loci and low genetic divergence makes accurate genetic assignment and descriptions of introgression and shared ancestry problematic (Allendorf et al. 2001; Vaha and Primmer 2006).

The wild baseline appears to contain evidence for hatchery introgression (Table 1, Figure 2). It had an estimated proportion of hatchery admixture (i.e., mean STRUCTURE-based q_i) of 0.35, and 25% of the individuals had q_i estimates greater than 0.50 (i.e., they are estimated to have more hatchery ancestry than wild). In addition, variance in q_i values among individuals was high in comparison to the hatchery baseline, lending further support to the presence of introgression. Only 4 and 14% of pairwise locus comparisons were significant (P<0.05) in the three collections, suggesting that introgression from the hatchery has occurred for many generations (e.g., Allendorf et al. 2001). Nevertheless, low genetic divergence between the hatchery and wild makes it difficult to discern introgression from shared ancestry.

Estimated proportions of hatchery admixture in the juvenile samples from upper and NF Eagle Creek ranged from 0.33 to 0.70 (Figure 3). Upper Eagle Creek had the highest proportion of admixture with mean q_i=0.68(SD=0.33) and 0.70(SD=0.33) in 2010 and 2011, respectively. Mean q_i in NF Eagle Creek was 0.56(SD=0.36) and 0.33(SD=0.34) in both years, respectively. Linkage disequilibrium was relatively low in NF Eagle Creek in 2011 and in upper Eagle Creek in 2010 and 2011; proportions of loci in significant linkage disequilibrium ranged from 0.06 to 0.09. Low linkage disequilibrium may indicate the presence of a 'hybrid swarm' between wild and hatchery fish. In contrast, 34% of pairwise locus comparisons were significant in NF Eagle Creek in 2010, possibly indicating 'recent' or ongoing hatchery influence and admixture.

Hatchery admixture in wild populations in the Eagle Creek basin appears dynamic. In particular, q_i distributions appear to fluctuate within creeks substantially through time (Table 1, Figure 3). For example, hatchery influence is significantly higher in upper Eagle Creek than in NF Eagle Creek in 2010 and 2011; however, in 2005 the influence was higher in NF Eagle Creek. Thus, it is important to interpret the genetic impact of the hatchery in the context of the timeframe in which it was sampled.

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

Table 1. Summary statistics for the hatchery and wild baselines and for juvenile samples collected from throughout the Eagle Creek basin. q_i is STRUCTURE-based proportion of hatchery admixture within an individual: q_i=1.00 and q_i=0.00 represent hatchery and wild fish, respectively. LD is the proportion of pairwise locus comparisons in significant (P<0.05) linkage disequilibrium.

Sample	Collection Year	N	Mean qi	LD
Hatchery	2005	55	0.91(0.09)	0.11
Hatchery	2006	48	0.92(0.06)	0.17
Hatchery	2007	60	0.89(0.17)	0.04
Wild	2005	42	0.35(0.31)	0.04
Wild	2006	29	0.24(0.32)	0.06
Wild	2007	29	0.47(0.36)	0.14
lower Eagle Creek	2005	50	0.17(0.24)	0.08
lower Eagle Creek	2006	29	0.34(0.34)	0.08
lower Eagle Creek	2007	24	0.47(0.34)	0.07
NF Eagle Creek	2005	50	0.48(0.34)	0.24
NF Eagle Creek	2006	70	0.38(0.37)	0.14
NF Eagle Creek	2007	47	0.42(0.29)	0.09
NF Eagle Creek	2010	51	0.56(0.36)	0.34
NF Eagle Creek	2011	49	0.34(0.34)	0.10
upper Eagle Creek	2005	50	0.24(0.32)	0.09
upper Eagle Creek	2006	94	0.36(0.37)	0.35
upper Eagle Creek	2007	65	0.71(0.32)	0.18
upper Eagle Creek	2010	52	0.68(0.33)	0.06
upper Eagle Creek	2011	48	0.7(0.34)	0.06

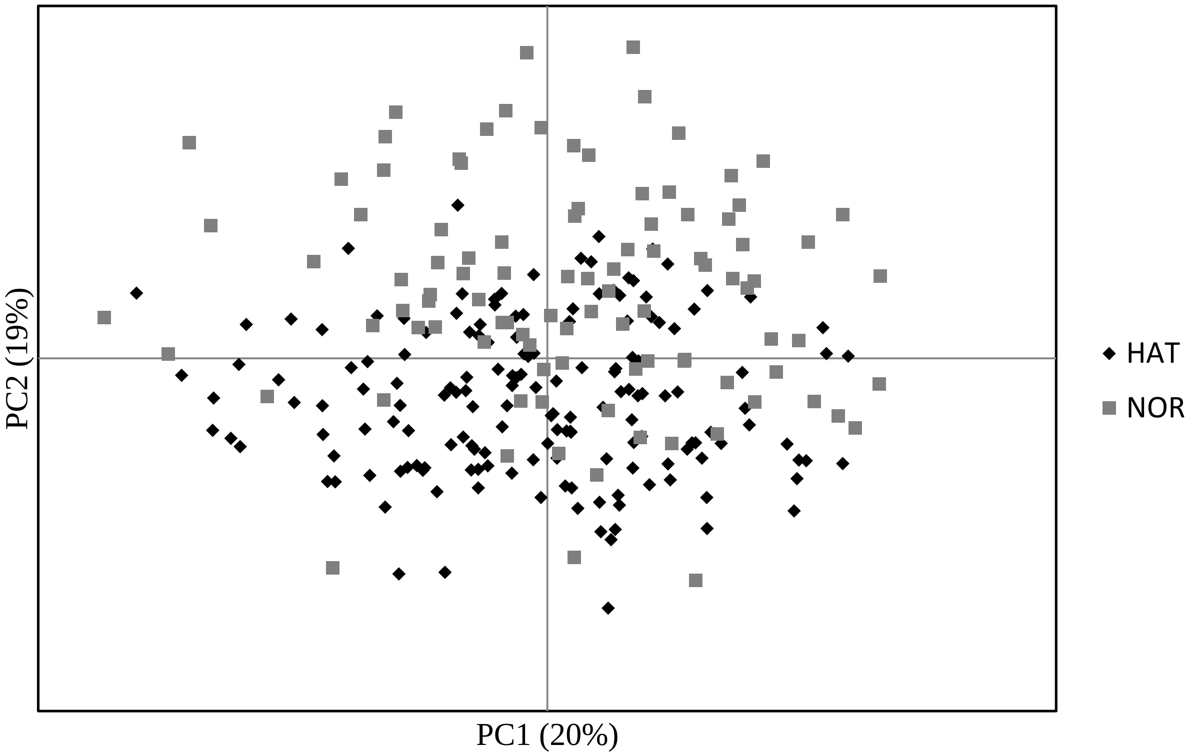
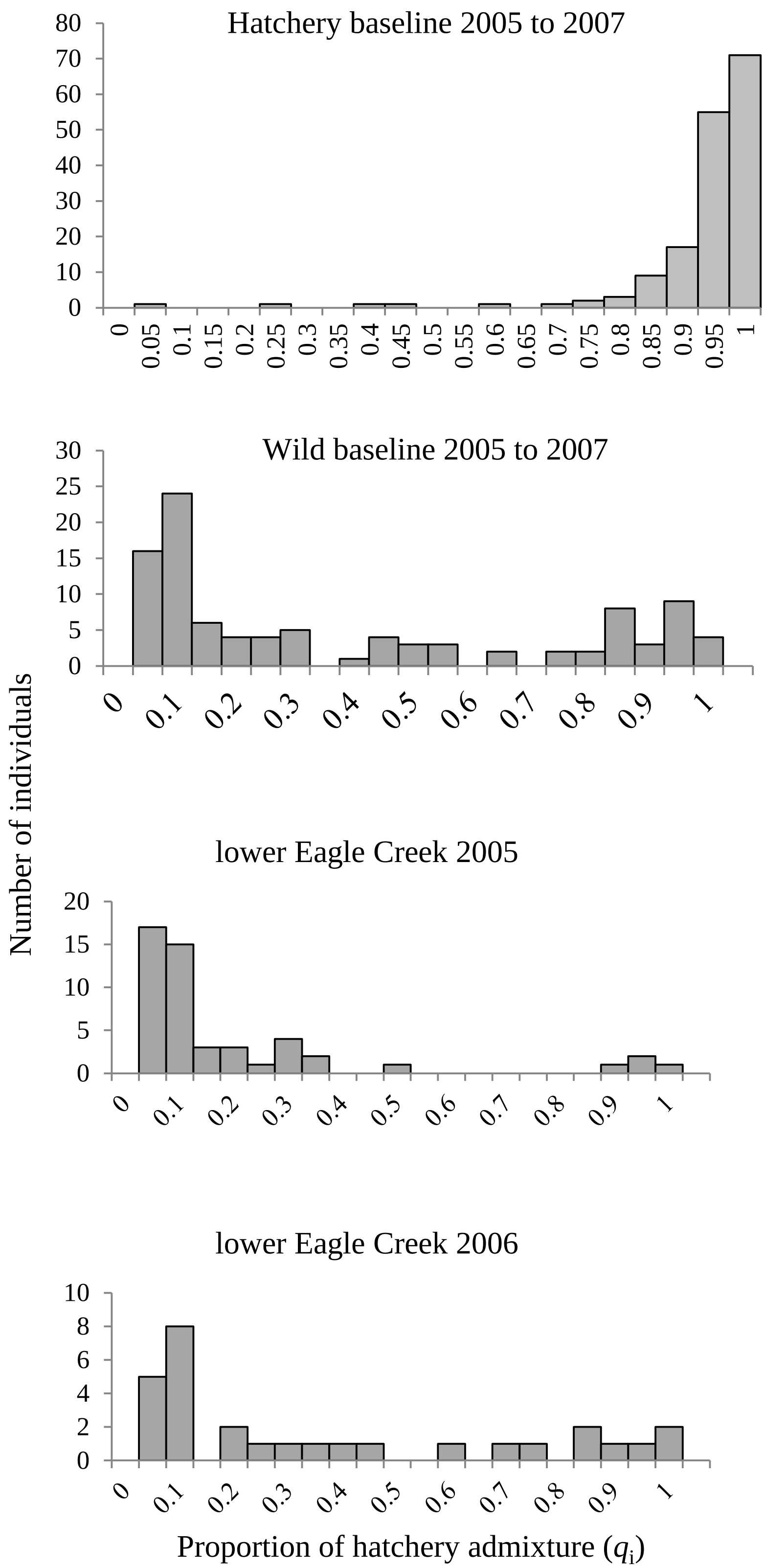
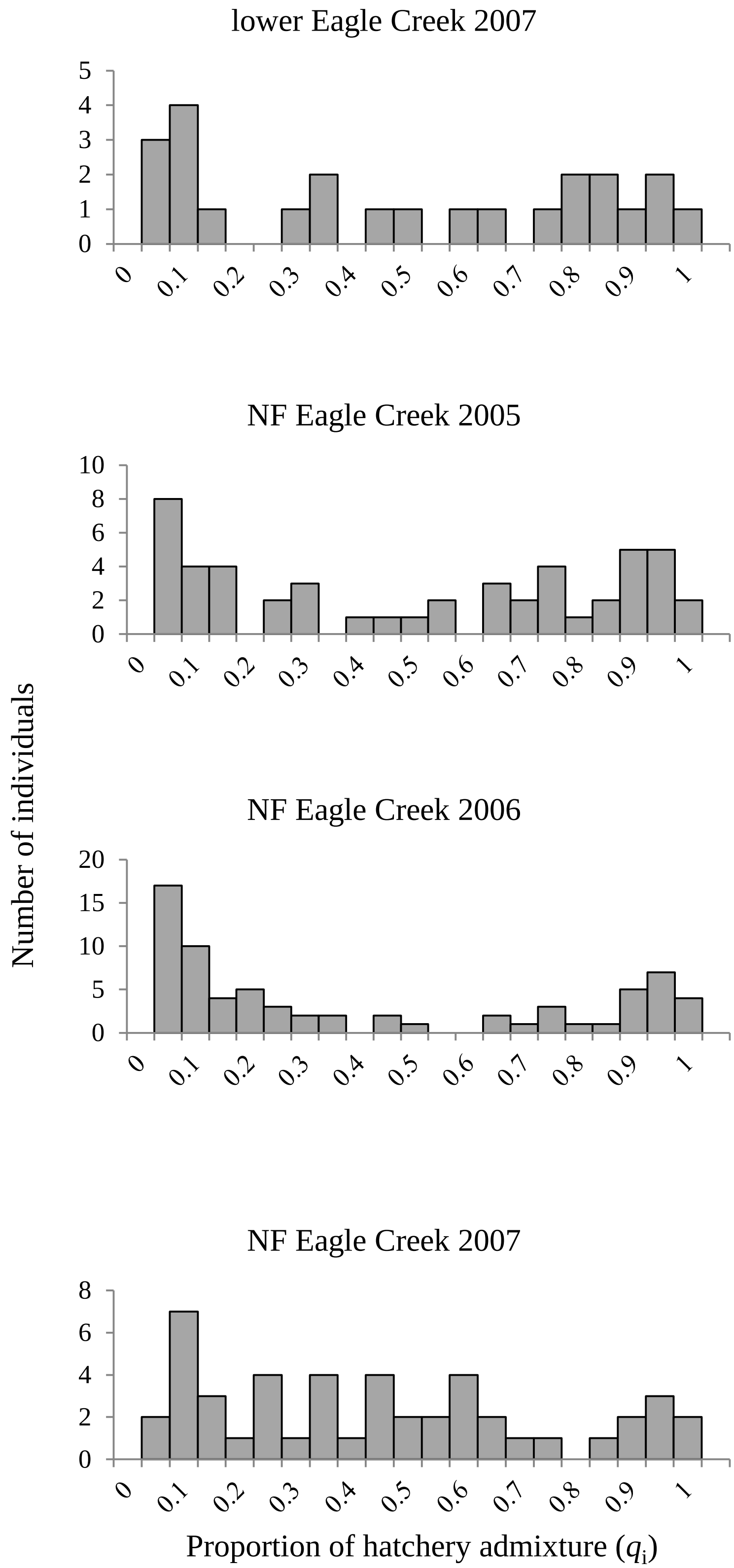
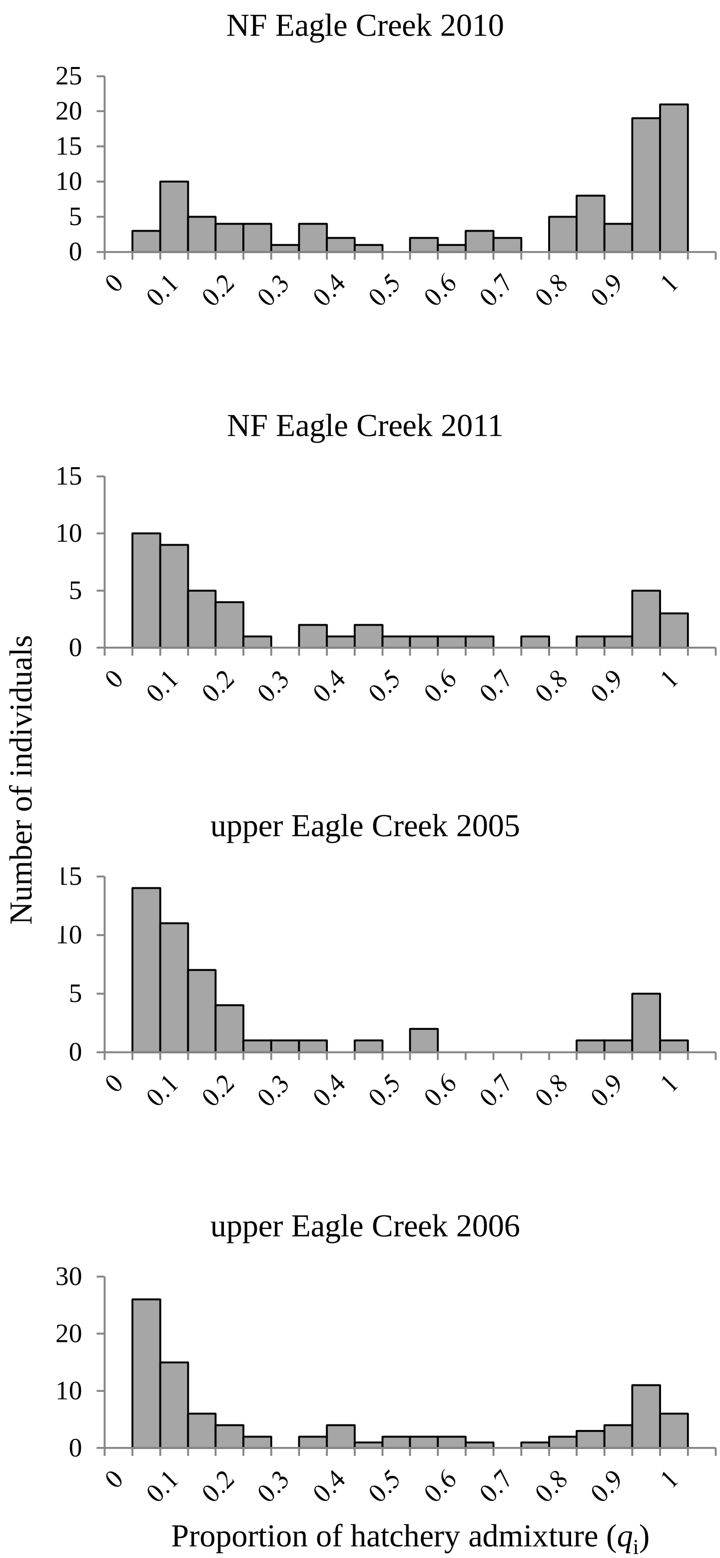


Figure 1. Plot of the first two principal coordinate scores derived from individual-based variation at 15 polymorphic microsatellite loci. The percentage of variation attributable to each component is shown for each axis.


Hatchery baseline 2005 to 2007
80
70
60
50
40
30
20
10
0
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
0.65
0.7
0.75
0.8
0.85
0.9
0.95
1
Wild baseline 2005 to 2007
30
25
20
15
10
5
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Number of individuals
lower Eagle Creek 2005
20
15
10
5
0
lower Eagle Creek 2006
10
8
6
4
2
0
Proportion of hatchery admixture (q_i)


lower Eagle Creek 2007
5
4
3
2
1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
NF Eagle Creek 2005
10
8
6
4
2
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Number of individuals
NF Eagle Creek 2006
20
15
10
5
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
NF Eagle Creek 2007
8
6
4
2
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Proportion of hatchery admixture (q_i)


NF Eagle Creek 2010
NF Eagle Creek 2011
upper Eagle Creek 2005
upper Eagle Creek 2006
Number of individuals
0
5
10
15
20
25
30
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Proportion of hatchery admixture (q_i)

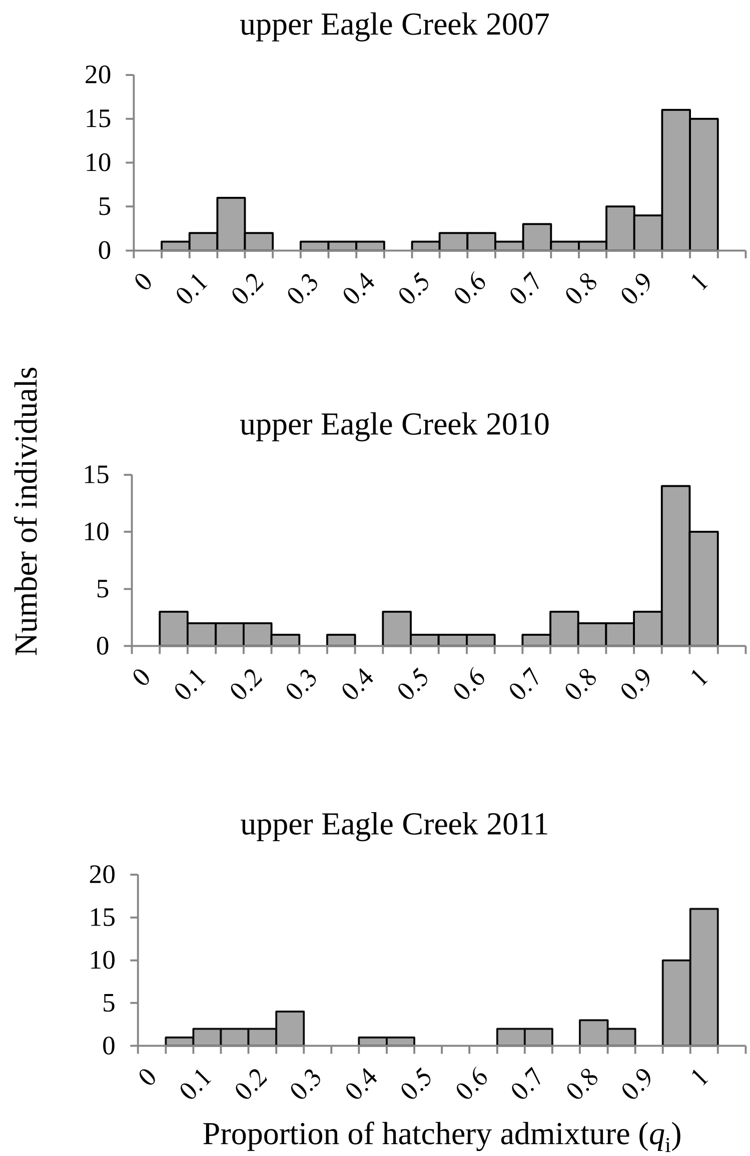


Figure 2. Distributions of the STRUCTURE-based proportions of hatchery admixture in juvenile *O. mykiss* samples collected in the Eagle Creek basin. q_i=1.00 and q_i=0.00 represent hatchery and wild fish, respectively.

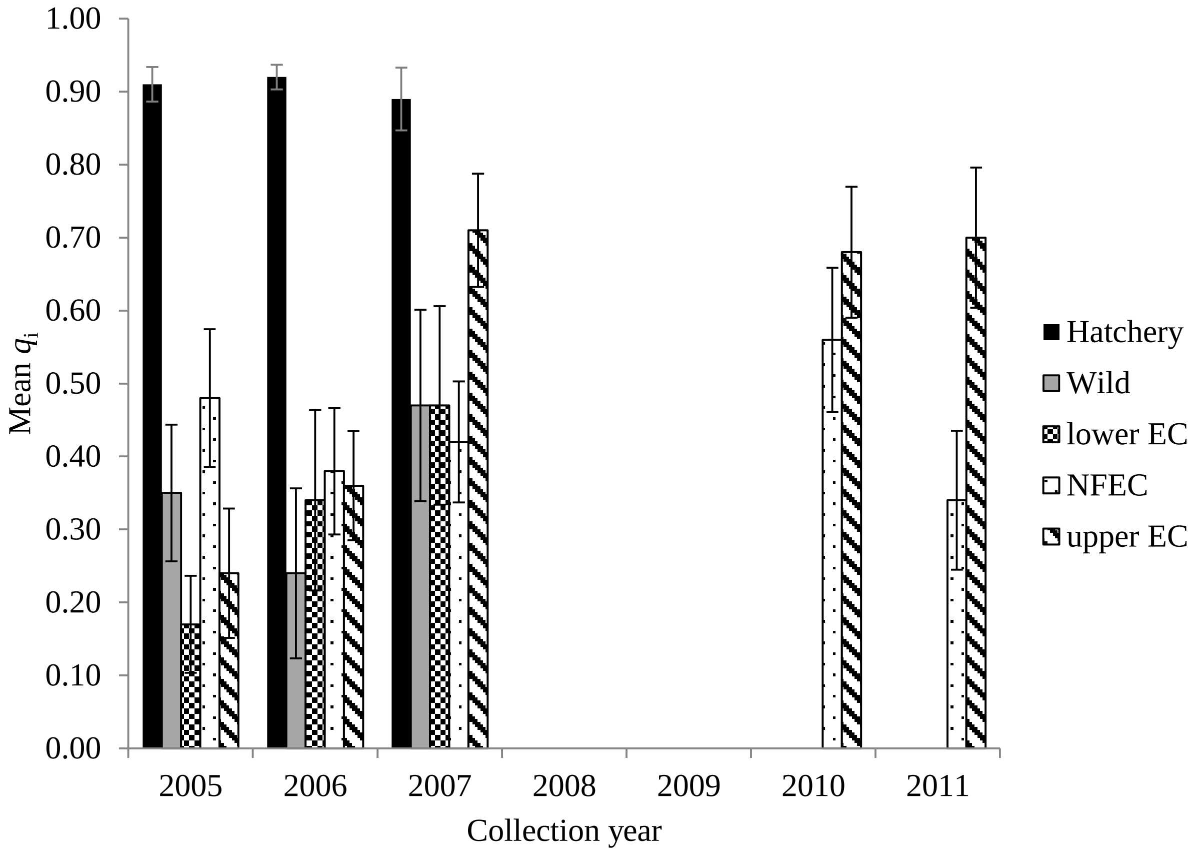


Figure 3. Mean and 95% confidence intervals for STRUCTURE-based proportion of hatchery admixture estimates (q_i) within juvenile collections of *O. mykiss* sampled in the Eagle Creek basin.

Literature cited

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology & Evolution 16(11):613-622.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics 1:47-50.

Kavanagh, M., and coauthors. 2009. Ecological and Genetic Interactions between Hatchery and Wild Steelhead in Eagle Creek, Oregon. CRFPO Final Report.

Matala, A., Ardren, W., Kavanagh, M., Brignon, W., Hogle, J., and Olson, D. 2007. An evaluation of natural productivity and genetic interactions between a segregated hatchery stock and wild steelhead trout (*Oncorhynchus mykiss*) in Eagle Creek, Oregon. AFTC Final Report.

Peakall, R. O. D., and P. E. Smouse. 2006. genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6(1):288-295.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155(2):945-959.

Vaha, J.-P., and C. R. Primmer. 2006. Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. Molecular Ecology 15(1):63-72.

Appendix B:

Double Rainbow by Tess McBride, U.S. Fish and Wildlife Service, *Reflections on Fisheries Conservation, Eddies, Spring 2011*, Volume 4 (1) 22-23.

By Tess McBride

Double Rainbow

Bill Brignon, a fish biologist with the Columbia River Fisheries Program located in Vancouver, Washington, hands down a pair of boots and waders from the bed of his truck. After jumping into them I feel like a kid geared up in ski boots and a snow jumper nervously trudging out for my first mountain experience. This isn't just because I'm nervous, but also because it feels awkward to lug such large and heavy boots on dry land.

Brignon speaks into his walkie talkie as he swats away branches lingering in his path while we make our way down to the creek. I try to avoid the flying foliage as I get used to walking in foreign shoes. Putting on somebody else's shoes for a day is exactly what I'm here to do, both physically and figuratively, in order to find out what it's like to do fisheries work hands-on.

Today I'm pretending to be a fish biologist, which already appears to be as demanding physically as I assumed it would be intellectually. Brignon and fish biologist Maureen Kavanagh invited me to participate in the tagging procedure they perform on Eagle Creek.

"We're comparing to see if the hatchery fish that are spawning here are affecting the wild fish here, and if so what can we do to lessen that," Kavanagh explains of the project. For some reason a large portion of hatchery fish are choosing to spawn in Eagle Creek and are not returning to their hatcheries of origin, which is traditionally what steelhead do. "It's really important that we manage the hatcheries in a way that we don't harm the wild population," Kavanagh said.

Chee Xiong, an Oregon State University fisheries student, lays out a scale and a row of syringes on a board that he places on his lap. Each needle contains a tag that will be inserted in the belly of a steelhead. Xiong is working with the tagging team under the Student Temporary Employment Program (STEP). This program provides college students paying experience in their academic fields, while continuing with school, and they learn about the U.S. Fish and Wildlife Service.

Xiong places a motionless fish next to a ruler and then on a scale. "Getting PIT-tagged," he declares while reaching for a needle and turning the fish on its back in his hand. Xiong has a serious expression on his face as he gently but swiftly inserts the needle into the fish and pushes the tag into its belly.

We soon see the netting team who just zigzagged across the creek scooping up steelhead that float to the surface after being electroshocked. Electroshocking is a non-lethal process that temporarily stuns the fish so that it can be caught. Casey Callahan, a Washington State University STEP student, has the duty of electroshocker this round; holding out a long pole with a metal ring on the end into the creek, the other end attached to a large plastic box on his back, which resembles a prop from *Ghostbusters*. I'm later informed the team refers to this as the "fish capacitor," referencing the DeLorean-turned-time machine from *Back to the Future*, which Doc Brown calls the "flux capacitor."

Callahan's team includes two netters and a fish carrier; Brian Davis, an Oregon State University STEP student; Trevor Conder, a fish biologist; and Sheila Davis, a seasonal fish biologist. The group is laughing and giving each other high-fives as they set down their gear and reflect on the successes and failures of the previous run. Both are laughed about; including a Spring Chinook sighting and Callahan's near fall with the electroshocking equipment on his back.

"Casey's going to have to hear about that one for a while," Brignon joked when we heard the commotion of the incident downstream. He was right. It's obvious these team members have a great vibe, and are both serious about the work they do and excited to be out in the field. This is why I'm thrilled to join the next round of fish wranglers as a netter.

I try my best to keep up with the team while scouting ideal placement for fish scooping. Conder, the electroshocker of this round, lets us know of his next plan of attack and we gather near a section of large rocks. As soon as the shiny bellies of two steelhead emerge I dive in and catch them both.

"Double rainbow!" the group shouts in reference to a YouTube video of an overly enthusiastic man who spots two rainbows, which they've incorporated into a reoccurring inside joke. I begin to feel pretty good about my loot, until Brian Davis scoops down and out does me. He's definitely the pro of our group; knowing where to stand, netting at lightning speed and scooping with a twist of the wrist that snaps the fish out of the water before I even spot them. As I begin to focus on the thrill of the catch I stop paying enough attention to my feet, resulting in a few slips and spills.

"Your style reminds me of Casey," Sheila Davis jokes, "willing to risk life and limb for a catch." Perhaps I am a bit wobbly, but I'm just determined to do my share. By the time we meet up with the tagging group we've filled two buckets with fish. I feel pumped

Tess McBride/USFWS

Each syringe is loaded with tiny tags that will be inserted under the skin of young steelhead. The data coming from tag returns will help guide fishery managers with future decisions.

classroom for his senior year, noting how useful it is to have experience doing what his professors are lecturing about.

"I like handling fish and being outside. It's pretty exciting to see what we net up," he said. One thing Xiong mentions he didn't expect from this summer was the intensity of a day's work. Right on cue Brignon announces it's time to pack up and hike up "Heart Attack Hill." If at all doubtful before, I'm now sure I'll experience the exhaustion Xiong spoke about.

from the excitement of the run, but also ready for some lunch, which we sit down along (and in) the creek to enjoy.

After lunch I join the new tagging group. We charge up the creek and set up our station on a small bank about 10 feet above the creek. Not much room to work, but we're able to make do by strategically placing our supplies around Callahan, who sits on a rock and prepares to do the tagging this round. Callahan, who will be a sophomore next year, is the youngest of the group and is in the middle of his first summer working as a STEP student.

"I went into this thing and soaked up a million pieces of information and got some new hobbies," he said of his experience working in the field. "Jobs in the outdoors are really cool," he adds. While Callahan enjoys his work in the field, he still plans to study pre-med in school.

"Pre-med also happens to be Casey's nickname out here," Sheila Davis chimes in. Callahan laughs and nods his head in agreement while splashing a handful of water on the ruler he prepares to place a fish on.

At our final tagging site I get a chance to talk more with Xiong. He predicts his work in the field will give him a one-up when going back into the

After panting our way up to the cars, the gang invites us along their ceremonial end of the day activity; getting Slurpees at 7-Eleven. We tag along (since we're such good taggers at this point), and after creating our frozen beverages we form a circle in the parking lot to compare flavors and stacking techniques.

When we drive away the group is still laughing in a circle. It's pretty clear this team's strong dynamics lives in and out of the creek. One day down, 200-plus fish tagged, a handful of Slurpees consumed, a couple of references to classic '80's movies made, and a great attitude about tomorrow still going strong. Double rainbow indeed. ✦

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